Temporal Currency:
Life-history strategies of a native marine invertebrate increasingly exposed to urbanisation and invasion

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Abstract

Biological invasions pose a serious threat to biodiversity world-wide. Through various means, such as competition or predation, invaders can radically change species composition and the functioning of native ecosystems. Even though our understanding of the mechanisms underlying invasion success is improving, there is still a lack of knowledge on the response of native species under pressure from invasion. This study adds to existing knowledge on the responses of a native species to invasion by non-indigenous species.

Pyura pachydermatina is a native ascidian in the southeast coast of New Zealand currently under pressure from increased urbanisation and invasion by other ascidian species. The reproductive strategies employed by *P. pachydermatina* are investigated and the role of these strategies to increase its resistance to invasion are assessed. A population study on the status of *P. pachydermatina* around the Banks Peninsula was carried out in Camp Bay, Pigeon Bay, and Wainui. Spawning experiments using *P. pachydermatina* and gonad histology were done regularly during the one year study period to assess its ability to self-fertilise and determine its reproductive period. In addition, predation experiments were carried out to assess the susceptibility of *P. pachydermatina* early life stages to two amphipod predators.

The surveys indicated that the populations of *P. pachydermatina* in the three sites are different from one another. Wainui has on average the largest individuals of *P. pachydermatina* and Camp Bay, the smallest. Abundance of *P. pachydermatina* was highest in Pigeon Bay and lowest in Wainui. The three life stages of *Pyura pachydermatina*; recruits, juveniles, and adults, were present in all sites at all seasons. The spawning experiments confirmed the species' ability to self-fertilise and that it has a year-round spawning period.

The two amphipod predators, *Jassa marmorata* and *Caprella mutica*, were efficient in consuming the egg and larval stages of *P. pachydermatina*, but did not feed on the settlers.

Year-round reproduction and the ability to self-fertilise potentially give *P. pachydermatina* increased resistance to the effects of urbanisation and invasion. This population study suggested that the species is thriving around the Banks Peninsula. This, combined with previous studies on the non-indigenous ascidian *Styela clava* that stated the static or declining populations of the potential invaders, gives a positive outlook for the native species for the future. I suggest the use of genetic techniques to assess, in more detail, the population structure and dispersal potential of this native species. I also suggest constant monitoring of native species is required to keep up to date with the current status of the species, which will in turn help management decisions should regional spread of the Lyttelton *S. clava* invasion occur in the future.

Chapter 1

General Introduction

Understanding the interactions between native communities and non-indigenous species is essential to determining whether invasive species will become established and impact the native species. Invasive species of both terrestrial and marine nature have been studied extensively in the past few decades (Carlton 1989, Spanier & Galil 1991, Blossey & Notzold 1995, Ruiz et al. 1997), and continue to be a primary focus of general ecology at the present time (Lankau 2012, Rius & Shenkar 2012, Yamanishi et al. 2012). Comparatively, few studies have been done on native species at localities where biological invasions are prevalent, although there has been an increase in recent years (Edgell & Neufeld 2008, Shenkar & Loya 2008, Shinen & Morgan 2009). Consequently, our understanding of the mechanisms underlying successful invasions is rapidly improving, but our knowledge of the response of native species under pressure from invasions is still lacking.

In ecology, the adjectives used to describe concepts or theories have to be properly defined, in order to avoid confusions or different interpretations (Colautti & MacIsaac 2004). In my study, a non-native species becomes *invasive* when it largely outnumbers the native species

and has lead to a change in the ecosystem. For example, the solitary ascidian *Ciona intestinalis* is an invasive species because it has been shown to depress local species diversity and change sessile community composition in San Francisco Bay (Blum et al. 2007). The term invasive species is not to be confused with the term *non-indigenous species* (NIS). NIS have different evolutionary histories to those of the native species in the community to which they were introduced, and their movement into these new areas has been aided by anthropogenic activities (Colautti & MacIsaac 2004). NIS are found in areas outside of their natural geographical distribution, but do not necessarily have to be invasive, although they may have the potential to be invasive in the future. For example, the western Pacific grapsid crab, *Hemigrapsus sanguineus*, is an NIS and has the potential to alter the community composition and become invasive in the mid-Atlantic coast of North America (Gerard et al. 1999).

My study adds to existing knowledge on the responses of native species to invasion by non-indigenous species. The subject of this study is a native ascidian species, *Pyura pachydermatina*, currently under pressure from invasion by another ascidian species and I assess the mechanisms it may use to cope with the impacts of invasion.

1.1 Marine urbanisation and invasion

Invasive marine species have been a major threat to biodiversity, marine industries, and human health since humans started travelling in ships (Bax et al. 2003). It has been estimated that at any given time, up to 10,000 different species, are being transported around the world in ballast water tanks alone (Carlton 1999, Mooney & Cleland 2001, Bax et al. 2003), and this trend is likely to increase. Other vectors of invasive marine species include ships' hulls,

anchor chains, aquaculture equipment, and sea-chests (Carlton 1989, Ruiz et al. 1997, Coutts et al. 2003, Dijkstra et al. 2007a, de Barros et al. 2009). Transported alien species face a number of challenges during transport and they do not always survive. Environmental conditions and available niches in the new localities are often unsuitable to sustain individuals and populations of NIS. While many alien species establish themselves in new areas and integrate with the local biota, a number of them became dominate and outcompete the native flora and fauna (Bax et al. 2003).

Urbanisation is a major factor that contributes towards the increasing trend of NIS establishments in new areas (Floerl et al. 2004). While shipping largely facilitates the transport of a species; the ports or harbours at the ships' destinations act as recipients for the species (Castilla et al. 2004). The ever-expanding size and number of these urban facilities provide increasingly more suitable environments, thus further supporting the establishment of NIS in these areas (Connell & Glasby 1999, Holloway & Connell 2002, Bax et al. 2003). For example, the Port of Lyttelton has many man-made structures like pilings and pontoons that provide novel habitats with little competition for incoming NIS (Nutsford 2010, Webber 2010). Apart from ports and marinas, other man-made projects can contribute to marine biological invasions. A well-known example is the Suez Canal which broke down the terrestrial barrier and connected the Mediterranean Sea with the Red Sea. Since 1869, the completion of canal construction initiated an on-going process of marine introductions, generally from the Red Sea into the Mediterranean Sea (Galil 2009). The magnitude of these introductions of species and their impacts are of increasing concern. For example, more than half of the known non-native fish species in the Mediterranean introduced via the Suez Canal have established large populations (Goren & Galil 2005). In addition, some of the non-native invertebrates (e.g., the crab *Charybdis longicollis* and the jellyfish *Rhopilema nomadica*)

have been defined as pests due to the economic damage they have caused to fisheries and tourism (Spanier & Galil 1991, Lotan et al. 1994).

Although the impacts of marine invasions are usually negative, they can also be neutral or even positive (Castilla et al. 2004). In the Bay of Antofagasta, Chile, the non-indigenous ascidian *Pyura praeputialis* has been shown to alter the local intertidal ecology by outcompeting the native mussel populations. However, at the same time, it was also described as an ecosystem bioengineer NIS, providing habitat for 116 species of macroinvertebrates and algae (Castilla et al. 2004).

1.2 Successful invasion and establishment of populations

There are many factors affecting the success of newly arrived NIS. These factors can be divided into two general groups which are invasiveness and invasibility. Characteristics of invasiveness include the ability of the species to establish in, spread to, or become abundant in new communities; while characteristics of invasibility involve the susceptibility of habitats to the establishment and subsequent proliferation of invaders (Colautti et al. 2006, Leung & Mandrak 2007).

When reviewing the recent literatures on invasiveness, Colautti *et al*. (2006) found that propagule pressure was a significant factor and was positively associated with invasiveness. The concept of propagule pressure can be simplified into two main parts. Propagule pressure can refer to the measure of number of individual NIS arriving in a new area and the frequency of those arrivals (Holle & Simberloff 2005). This aspect of propagule pressure is predicted to increase following the trend for increasing ship traffic. The other part of

propagule pressure involves the ability of the initial NIS individuals to reach reproductive maturity and compound the effect of more incoming NIS by releasing offspring of their own (Lockwood et al. 2005). Underlying this aspect of propagule pressure is a series of life-history traits which are important in establishing the degree of invasiveness of a species. Sans *et al*. (2004) examined the various life-history stages between invasive and native shrubs in western Europe. They found that seedling emergence, seedling establishment, flowering time, growth, reproduction, and competitive ability for resources differ between the invasive and native species. The alien species were markedly more competitive than the native and suppressed all of the native species life-history stages. However, the competitive ability of the native species did emerge when resources, in this case nutrients and water, were added (Sans et al. 2004). In the marine environment, many of the transported NIS are invertebrates with the ability to produce large numbers of offspring and grow relatively quickly, leading to high propagule pressure (Davis & Davis 2007, Gittenberger 2007, Lambert 2007). Apart from propagule pressure, other characteristics of invasiveness include physiological tolerance, body size, growth rate, lifespan, and many more. How each of these characteristics contributes towards invasiveness depends on each species (Colautti et al. 2006).

The invasibility of NIS-recipient environments plays a large role in determining the degree of successful progression from NIS to invasive species (Sakai et al. 2001, Colautti et al. 2006, Osman & Whitlatch 2007). Many species cannot survive long voyages in the dark and dirty confinement of ballast tanks; for others, the environmental conditions at the port of discharge may not be suitable (Bax et al. 2003). If the local environmental conditions, such as water temperature, amount of light, and food availability are suitable for the NIS then it can survive and establish a population. For example, the adult stage of the crab *Carcinus maenas* has a wide tolerance to water temperature, but the larval stage have a narrower temperature

tolerance than the adults, thus limiting their potential spread to areas where the water temperatures are beyond their threshold of between 10°C and 22.5°C (deRivera et al. 2007). The native biodiversity also plays a role in that they either promote invasion by providing resources for NIS or resist invasion by predation or outcompeting NIS for resources. For instance, four invasive ascidians (*Botrylloides violaceus*, *Diplosoma listerianum*, *Styela clava*, and *Ascidiella aspersa*) in Long Island Sound, USA, dominate many of the harbour areas, but are not found outside in the more open areas as a result of predation on their juvenile life-stages by fish and various benthic invertebrates (Osman & Whitlatch 2007). Noonburg and Byers (2005) developed a model for the effect of predation in reducing the number of NIS. They went one step further with the model and included the interaction with the native species. They proposed the idea that by decreasing the number of NIS, the number of predators would increase, and if the predators prey on native species, then the number of native species would decrease as well. This idea is very dynamic and depends on the level of interactions between the predator and prey, which can either be NIS or native species or both; different predictions can be made for the outcome on the community biodiversity.

In summary, the mechanisms involved with NIS establishment and progression into invaders are often complex and integrative. Even though the elements of invasiveness and invasibility differ – propagule pressure is a function of time whereas invasibility is not, it is unquestionable that the interaction between the two is crucial in establishing a successful invasion (Leung & Mandrak 2007).

1.3 Ascidians

Ascidians, both the solitary and colonial species, have received great attention as marine invaders (Davis & Davis 2006, Blum et al. 2007, Bullard et al. 2007, Lambert 2007, Arsenault et al. 2009, Rius et al. 2009). The ascidians or sea squirts are a primitive group of sessile marine invertebrates that are efficient filter feeders of small particles suspended in the water column. Adult ascidians firmly attach themselves to hard substrates and produce numerous short-lived larvae that enable dispersal of the species over short distances (Svane & Young 1989). Thus, the appearance of ascidian species in areas far from their original geographic distribution is an indication of transport via anthropogenic vectors, usually ship's ballast water or hull fouling (Lambert 2007). A notorious example of how an invasive ascidian species can spread and affect the local community negatively is given by Arsenault *et al*. (2009) using *Styela clava*. In just three years during their study, they found that *S. clava* spread up to 12 km away from the initial detection area and the number of recruits they sampled had increased by more than 900-fold. As a result, this invasive ascidian has almost completely decimated the mussel farming industry in Prince Edward Island, Canada. In another study, Dijkstra *et al*. (2007b) found that the two-dimensional colonial ascidians (*Botrylloides violaceus*, *Diplosoma listerianum*, and *Didemnum sp.*) depressed the native biodiversity in the Gulf of Maine, USA, by dominating the available space during recruitment and also by inhibiting the recruitment of other fouling organisms by synthesizing allelopathic chemical compounds. Ascidians now dominate fouling communities in ports and marinas and are a very real threat to native ascidian communities outside these ports of entry.

1.4 Native ascidians as study organisms

The effect of NIS on native ascidians is not well studied; how they are impacted by invasion and urbanisation, and on the mechanisms they have to compete and ultimately resist invasions. Shenkar and Loya (2008) investigated a native and an invasive population of the solitary ascidian *Herdmania momus*. The differences in life history strategies employed by this species in the two separated populations were attributed to differential food availability, water temperature, currents, and wave exposure. Although all these factors are environmental, they strongly affect species' spatial distribution, body size, reproductive cycles, and symbiotic relationships with other organisms. Therefore it is crucial to understand the biology of the species and the local environmental conditions before investigating how the species might be impacted by change from invasion. One resistance mechanism that may be present in ascidians is chemical defences. Ascidians are known to be chemically rich and these chemical often deter predators (Stoecker 1980, Lindquist et al. 1992) or inhibit the recruitment of other fouling species (Dijkstra et al. 2007b). Solitary ascidians can increase their chances of successful establishment when competing with other species by having rapid growth, small-sized offspring, and short generation time to settle and start growing (Altman & Whitlatch 2007). My study investigates native ascidian reproductive strategies as potential resistance mechanisms against increasing pressure of invasion.

1.5 *Pyura pachydermatina* – a New Zealand native ascidian facing invasion pressure

Pyura pachydermatina (Herdman 1881).

Taxonomic status
Kingdom: Animalia
Phylum: Chordata
Subphylum: Tunicata
Class: Ascidiacea
Order: Stolidobranchia
Family: Pyuridae
Species: *Pyura pachydermatina* (Herdman 1881)

Commonly known as sea tulips or kāeo in the Maori language, *Pyura pachydermatina* (hereafter referred to as *Pyura*) (Figure 1.1) is not to be confused with *Pyura spinifera* (Quoy & Gaimard 1934) with which it shares the common name. The common name comes from its appearance which resembles the 'bulb' or flower of the tulip plant, attached to a long thin stalk. Its body is enclosed in a tough leathery tunic, from which the name tunicate is derived, in a variety of colours ranging from white, cream, pink, maroon, purple, or a combination of these. The tunic has a number of grooves that run longitudinally and sometimes has small wart-like protrusions known as tubercles (Figure 1.1). This tunic acts as an exoskeleton that gives protection and structural support to the living animal enclosed within. It is also a living tissue in that it grows together with the animal inside; ascidians are the only animals that do not exhibit molting of their exoskeleton as they grow (Ruppert et al. 2004). The long stalk which is *Pyura*'s attachment point can be as long as 1 metre in length. This makes *Pyura* one of the largest of all the solitary ascidians (Ruppert et al. 2004). Like all ascidians, *Pyura* is a filter feeder and has two siphons on the lateral side of its body to accommodate this feeding mechanism. The oral siphon is closer to the stalk and is used to uptake water, while the atrial siphon is located further away from the stalk and is used to expel water, excrete wastes, and

for spawning (Figure 1.2). There have been no specific study on the biology or ecology of *Pyura* in New Zealand, despite it having a Taonga status which is something akin to a national treasure to the Māori culture.

Several studies in Australia looked at the distribution and seasonal reproductive cycle of *Pyura pachydermatina* (Egan 1984a, b). Another one investigated the development and metamorphosis of *Pyura pachydermatina* larvae (Anderson et al. 1976). These studies provide the basic information on the biology and ecology of this species which are crucial for my overall study. The only other literature on this species to date is a biochemical study on the insulin-like material found in the digestive tract of this ascidian (Galloway & Cutfield 1988).

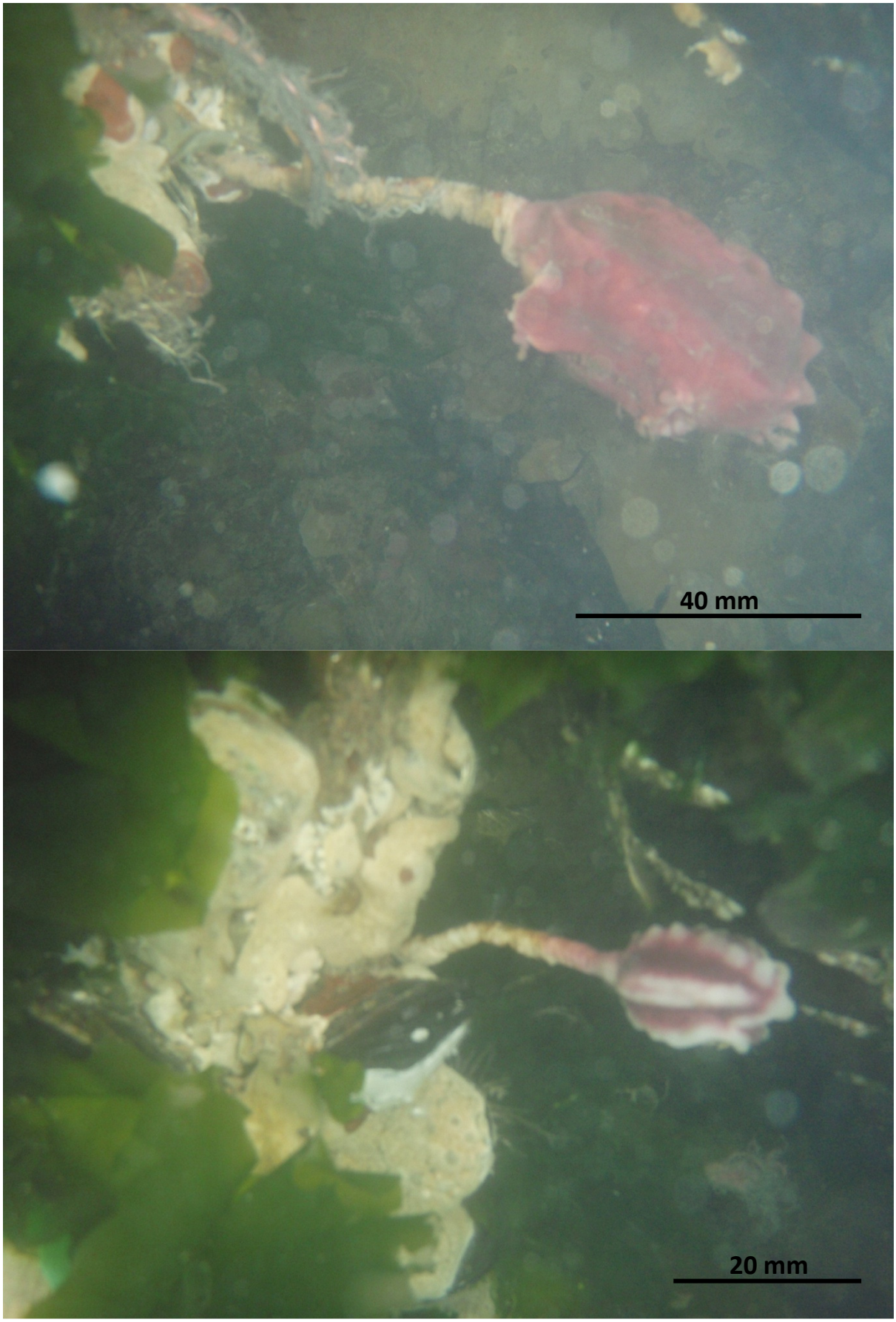


Figure 1.1. Photographs of *Pyura pachydermatina* underwater showing the different coloured tunics and the long stalk which it uses to attach to the substrate.

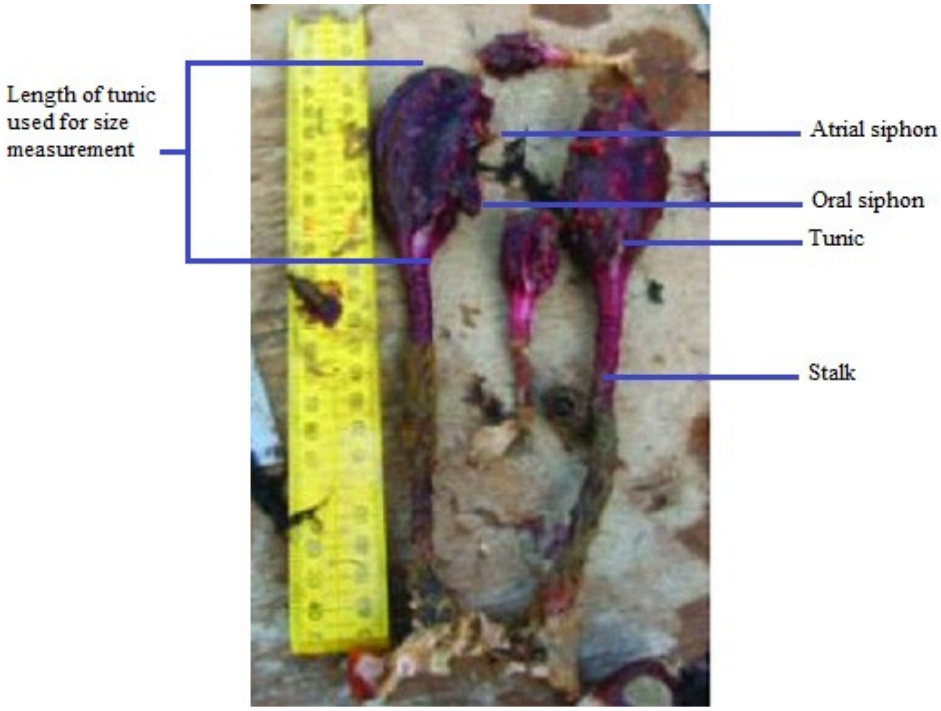


Figure 1.2. External anatomy of *Pyura pachydermatina* showing the positions of the two siphons, the main 'bulb' enclosed in a leathery tunic, and the long thin stalk. (Photo: www.biosecurity.govt.nz)

1.6 Banks Peninsula – local ecosystem and conditions

Banks Peninsula lies on the central east coast of New Zealand's South Island (Figure 1.3). The peninsula is generally exposed to a prevailing oceanic swell as this region marks the northernmost position of the Subtropical Convergence, also known as the Southland Front (Heath 1985). It is frequently exposed to high energy oceanic swells and storm waves. There are a number of variations of habitat structures on the Banks Peninsula, ranging from rocky reefs to sandy bottom area. *Pyura* is abundant on Banks Peninsula and occurs in the low-intertidal and subtidal to a depth of 15 metres (Schiel & Hickford 2001). In this region, the rocky shore sessile community is made up of fucalean algae (e.g., *Durvillaea antarctica*), laminarian algae (e.g., *Ecklonia radiata* and *Macrocystis pyrifera*), and sessile invertebrates such as bryozoans, mussels, ascidians, and sponges (Schiel & Hickford 2001).

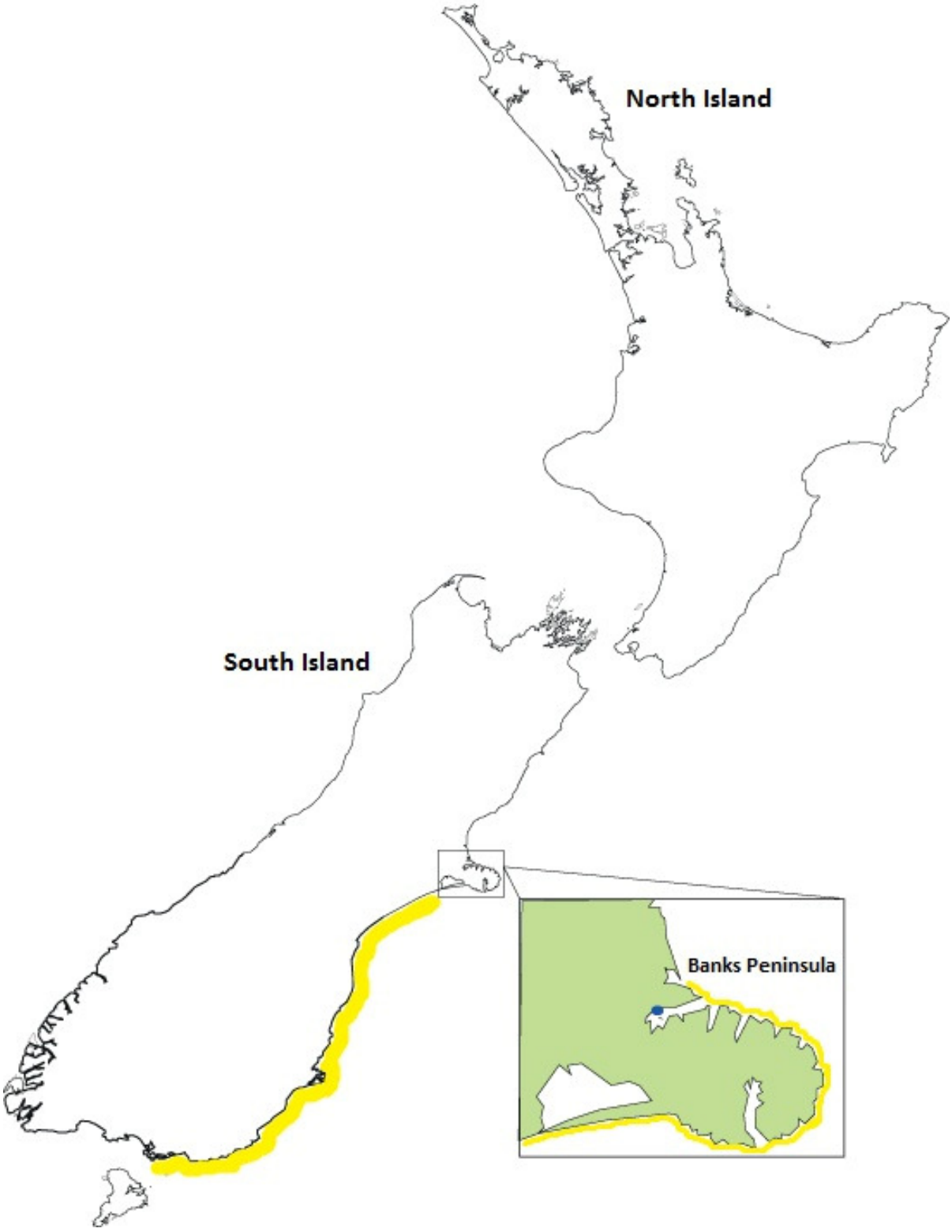


Figure 1.3. Map of New Zealand showing the localised distribution of *Pyura pachydermatina* (highlighted in yellow) on the south eastern coast of the South Island.

The water temperature in the region collected between April 2011 and February 2012 ranges from 8.2°C in the winter to 18°C in the summer (Figure 1.4). This data is consistent with the two decade average monthly water temperature at Taylor's Mistake (opposite side of the harbour from Camp Bay) where the recorded low was 8°C and the recorded high was 17°C (www.surf-forecast.com). Day length period in the Canterbury region varies between seasons as shown by Royal Astronomical data where measurements taken between sunrise and sunset were longest during summer (15 hours and 20 minutes) and shortest during winter (9 hours) (Royal Astronomical Society of New Zealand 1920). These longest and shortest day lengths are slightly different to the average longest and shortest day lengths during the summer (14 hours and 36 minutes) and winter (9 hours and 42 minutes) in the area of the earth between the Tropic of Capricorn (32.0°S) and the Antarctic Circle (150.0°S) (www.schoolsobservatory.co.uk).

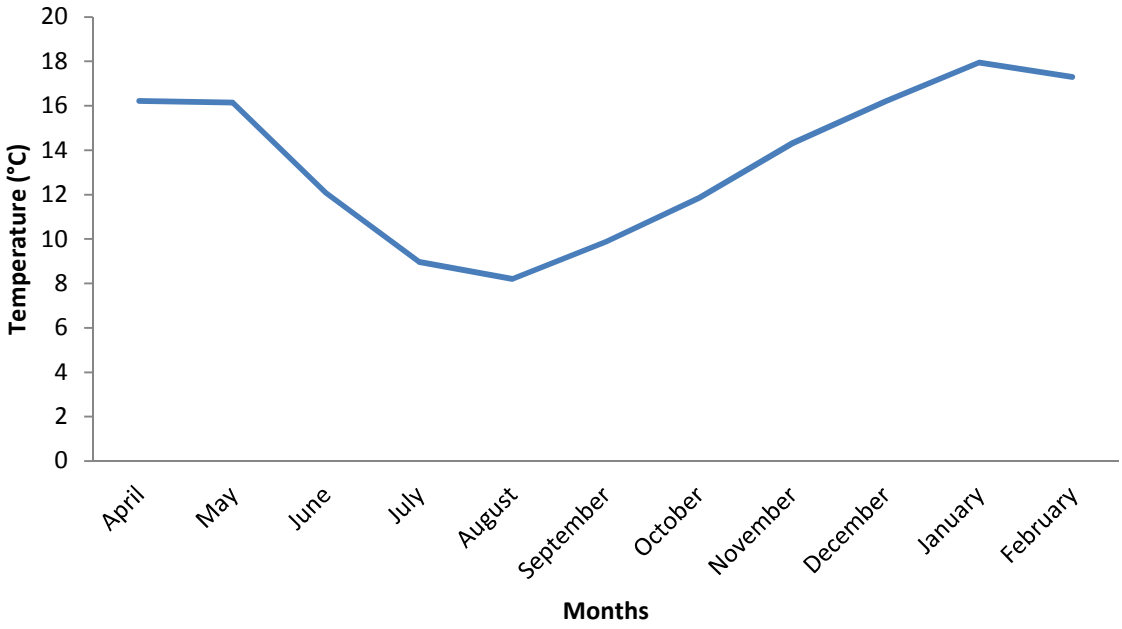


Figure 1.4. Mean monthly water temperature in Camp Bay, Banks Peninsula recorded from April 2011 to February 2012.

Banks Peninsula is also the location for the Port of Lyttelton, the primary deep-water port in the South Island. Many NIS have been reported in the Port of Lyttelton including *Ciona intestinalis*, *Undaria pinnatifida*, *Theora lubrica*, *Bugula flabellata*, and *Haliplanella lineata* among others (Inglis et al. 2006). In 2002 and 2004 during surveys conducted in the port and on ships' hulls, *Styela clava*, a New Zealand NIS and known invader worldwide, was reported (Gust et al. 2008). *S. clava* is known to have detrimental effects to native biodiversity and on the aquaculture industry (Bourque et al. 2007, Ramsay et al. 2008, Arsenault et al. 2009). In Prince Edward Island, Canada, it almost wiped out the entire area's aquaculture industry within a few years of first detection (Arsenault et al. 2009). Genetic evidence indicates that there have been multiple incursions of *S. clava* into New Zealand waters (Goldstien et al. 2010). Presently, *S. clava* distribution in Banks Peninsula is limited to the Port of Lyttelton and its neighbouring bays. Although the population in the port is either static or in decline, it appears that it may act as a stepping stone for the spread of this species outside of the port (Webber 2010). It has recently been found as far out as Governor's Bay (Goldstien pers. comm.).

1.7 Study outline and aims

The overall aim of this study is to investigate the life-history strategies employed by a native species, and assess how these strategies may increase resistance against increasing pressure from invasions. Particular emphasis is on the reproductive biology and its temporal aspects. It also describes the various features of current *Pyura* demography around the Banks Peninsula.

Chapter two, Demography of *Pyura pachydermatina*, describes the basic demographic features of *Pyura* in three sites around Banks Peninsula. It includes distribution, population

size structure, and abundance. These features were compared among the three study sites and also among the seasons during the one-year study period.

Chapter three, Reproductive Biology of *Pyura pachydermatina*, explores the potential ability of this species to self-fertilise. Contrasting information put the selfing capability of this species in doubt. A series of spawning experiments were set up to determine the reproduction and fertilisation of *Pyura* offspring throughout the year.

Chapter four, Reproductive Pattern of *Pyura pachydermatina*, looks at the temporal aspect of *Pyura* reproduction. Again, contrasting information clouded our understanding of the reproduction pattern of this species. Here I determined whether *Pyura* has a seasonal reproduction period or a year-round reproduction period. Spawning experiments from Chapter 3 and a series of gonad indices were used to paint a clearer picture of *Pyura*'s reproductive pattern.

Chapter five, Invasive Pressure on *Pyura pachydermatina*, involves predation experiments on early life stages of *Pyura*. This chapter also looks at the potential impacts of *Styela clava* on *Pyura* in Banks Peninsula, based on research of Nutsford (2010) and Webber (2010).

The final chapter presents a general discussion and evaluation of the demography and life-history strategies of *Pyura*. It describes the possible resistance of *Pyura* against *S. clava* invasion based on field data and experimental results.

Chapter 2

Demography of Pyura pachydermatina

2.1 Introduction

2.1.1 Background

Demography is crucial in the field of ecology as it provides an understanding of the basic processes occurring in populations of an organism. Demographic characteristics may also play an important role in the competition between native and invasive species. For example, traits promoting "weediness" such as high individual turnover, high propagule output, asexual, and seasonal reproduction are known to give invaders a competitive advantage in new environments (Sakai et al. 2001). In addition, invaders often evolve in response to novel biotic and abiotic conditions, which in turn drives the native species to evolve in response to the invasion (Sakai et al. 2001).

There are many studies which have focussed on the distribution and demography of invasive species (de Barros et al. 2009, Galil 2009, Hayward & Morley 2009, Rius & Shenkar 2012)

and their role in the reduction of native biodiversity, but fewer studies have been focussed on the distribution and demography of the functionally equivalent native species. On a more specific note, ascidians are notoriously known as one of the more capable phyla among marine invasive organisms (Bullard et al. 2007, Lambert 2007) and many native ascidians will be, if they are not already, faced with stiff competition from these invaders (Simoncini & Miller 2007, Shenkar & Loya 2008). Dijkstra *et al*. (2007a) and Dijkstra *et al*. (2007b) used settlement plates to follow the recruitment and establishment of four invasive ascidians in a harbour in New Hampshire and concluded that the structural morphology of the solitary non-indigenous ascidians was providing space for the native species and, therefore, not having a negative impact on the native biota, unlike the colonials which became the dominant space occupants and significantly reduced the abundance of other benthic organisms. Similarly Agius (2007) studied the role of five non-indigenous ascidians on the study organisms' growth, recruitment, and community composition; and concluded these species made up the majority of species richness and occupied as much as 80% of the local primary substrate. However, these and other studies do not take into account the demographic variability of the native assemblage. The distribution, size, age structure, reproductive period, and natural recruitment variability all influence the way a species responds to disturbances and competition over time (Jackson 1977, Shinen & Morgan 2009, Sandin & McNamara 2012), yet very little effort to date has been applied to understanding how these factors relate to the resilience of the native species against invaders (Holle & Simberloff 2005, Lankau 2012).

2.1.2 Abundance and Distribution of *Pyura pachydermatina*

Pyura pachydermatina (Figure 1.1), is distributed around the south coast of New Zealand, as well as the East coast of Australia. In New Zealand, *Pyura* is widely distributed on the rocky

shores of the South Island within the New Zealand Exclusive Economic Zone (NZEEZ) (Figure 1.3). It is particularly abundant on the east coast of the South Island, from the northern end of Banks Peninsula to the far south towards the Otago Peninsula and beyond (Schiel & Hickford 2001, Gordon 2009, Sanamyan 2010). This southern distribution may be influenced by the flow of the Southland current, which starts off from the west coast of the South Island travelling anticlockwise around the bottom of New Zealand and along the Otago-Canterbury coast until it reaches the Chatham Rise after which it is diverted eastward to the Chatham Islands, with partial flow around the Banks Peninsula heading north toward Kaikoura (Carter 2001). Some genetic studies done on the coastal marine organisms have shown that this region may create a "leaky" barrier to dispersal and population connectivity around Banks Peninsula. For example, the population genetic study on New Zealand native clam *Austrovenus stutchburyi*, showed that there was a distinct difference in the pattern of haplotype distribution north and south of Banks Peninsula in the South Island (Ross et al. 2012). Another population genetic study on three species of New Zealand native limpets also showed similar patterns of different haplotype distribution either side of the Banks Peninsula (Goldstien et al. 2006).

Rocky shore invertebrates live in a harsh environment with tidal influence dominating the vertical distribution on the platforms. In contrast, the longitudinal, or along shore, distribution of marine invertebrates is determined by a variety of other factors, such as active dispersal and space availability. The interplay between the life-histories, sea-surface currents, atmospheric interaction, biotic competition and abiotic factors, generally leaves most organisms of the rocky shore with a "patchy" or clumped longitudinal distribution as space becomes a limiting factor (Schiel & Hickford 2001). *Pyura* is known to have a patchy distribution. They can be found in large clusters of up to 50 or more individuals in one spot

(Figure 2.1) and occurs from the low intertidal to 15 metres depth (Egan 1984b, Schiel & Hickford 2001), but beyond this, very little is known about the demography of this species. Although *Pyura* are commonly found on the rocky shore where there is ample hard substrate for them to attach to, they can also be found attached to mussel shells or some macroalgae (e.g. *Macrocystis pyrifera*). They are attached in various orientations (pers. obs.); horizontally attached to the side of a rock, vertically attached to the bottom substrate, or vertically upside-down attached to the underside of an overhang.

Figure 2.1. Clusters of *Pyura pachydermatina* showing multiple individuals with the same stalk attachment points.

2.1.3 Chapter Objectives

This chapter focuses on the population demography of *Pyura* around the Banks Peninsula and attempts to answer the following questions:

- Are there any differences in the abundance of *Pyura* in the three study sites?
- Are there any seasonal differences in the abundance of *Pyura* during the one year study period?
- Are there any differences in the population size structure of *Pyura* in the three study sites?
- Are there any differences in the population size structure of *Pyura* seasonally throughout the year?

The above questions can be put into two sets of null and alternative hypotheses to be tested.

1. H_0 = The mean abundance of *Pyura* in the three sites and throughout the seasons are the same

   H_A = The mean abundance of *Pyura* in the three sites and throughout the seasons are not the same
2. H_0 = The mean size of *Pyura* in the three sites and throughout the seasons are the same

   H_A = The mean size of *Pyura* in the three sites and throughout the seasons are not the same

Assessing the abundance and size frequency of this species in its natural habitat is important to establish a basic understanding of the population biology. Together, these data can show if there is a single population around the local area or if there are in fact multiple populations. The three sites around the peninsula were chosen to represent populations to the north and

south of the peninsula, with Camp Bay and Pigeon Bay located on the northern side of the peninsula, and Wainui on the southern side (Figure 2.2).

2.2 Methods

2.2.1 Preliminary study

A preliminary study was carried out in January 2011 to assess the possible study sites and compare the effectiveness of two different sizes of quadrats. The sites were surveyed to assess the variability in distribution data and the number of quadrats required to capture this variability. Three natural sites were Camp Bay, Pigeon Bay, and Wainui; and the three man-made sites were the Port of Lyttelton, Magazine Bay Marina, and Akaroa Harbour Wharf. These sites were chosen based on a number of factors including ease of access and prior knowledge of presence of *Pyura*. The three study sites around the Banks Peninsula were the western side of Camp Bay (43°37'15"S,172°46'46"E), the eastern side of Pigeon Bay (43°38'7"S,172°55'30"E), and Wainui (43°49'46"S,172°54'33"E) (Figure 2.2). The original study design involved comparison between natural and man-made sites, which also put a cap on the depth of study to the top 1 metre of the subtidal zone, which is where the pontoons sit in the water. Due to the earthquake in February 2011, the urban sites were inaccessible and a decision was taken to drop that component of the study and focus on the natural sites. However, the preliminary study was completed by this stage and the results reflect some of these initial requirements.

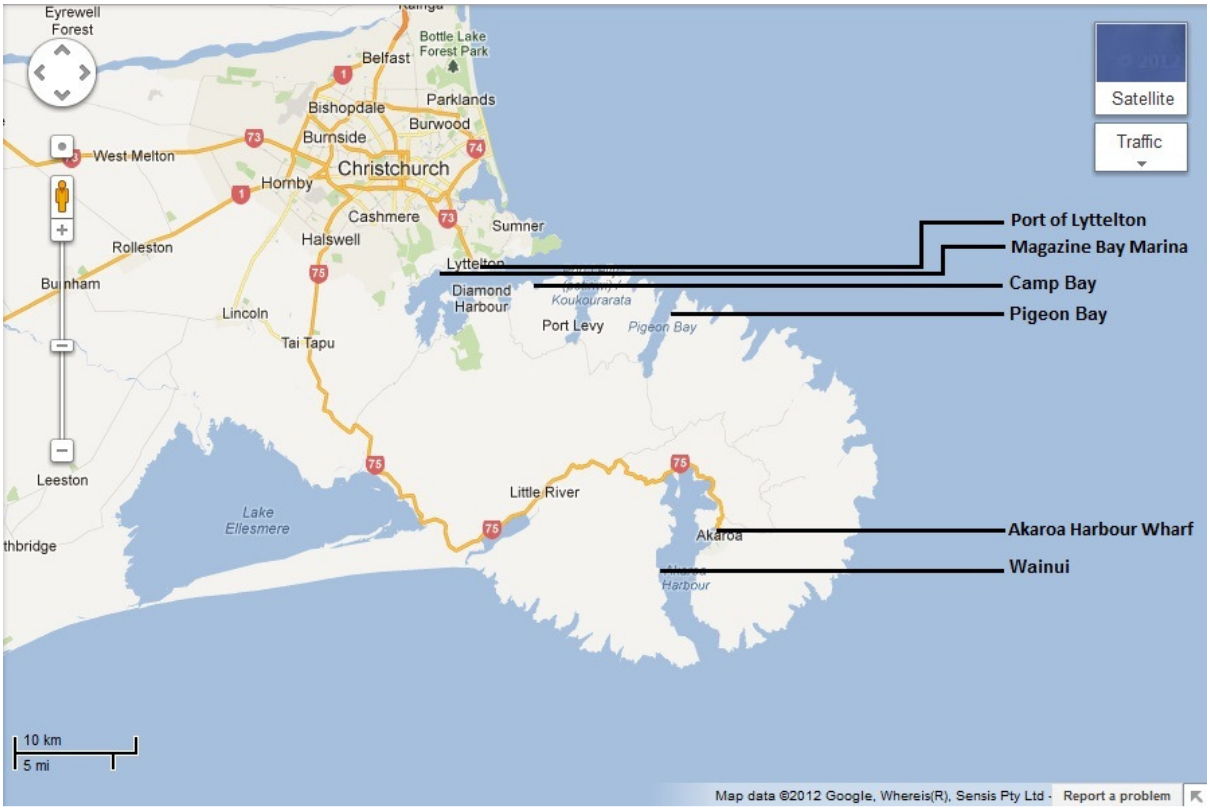


Figure 2.2. Map of the Banks Peninsula located on the east coast of the South Island of New Zealand showing the three natural study sites and the three man-made sites. (https://maps.google.co.nz)

Abundance was measured in the three study sites by means of quadrat sampling. Quadrat sampling allowed for a methodical sampling design (Figure 2.3) whereby the sampling area could be kept constant, while maintaining a random sample design. The larger (50 cm × 50 cm) quadrat was preferred to the smaller (25 cm × 25 cm) one for this study for its ease of handling in the water and was predicted to better capture the required data as *Pyura* has a patchy and sometimes clumped distribution (Morisita's Index=2.23). The applicability of the different quadrat sizes was assessed by plotting the cumulative mean abundance data obtained from sampling the same sites, using the same methods for both sizes in Camp Bay. Cumulative mean was used to find out the number of samples needed (in this case, the number of quadrat counts) to get an accurate estimation of population densities.

2.2.2 Abundance monitoring

Abundance data was collected on a monthly basis from January 2011 to February 2012, usually during spring tides, when the water level was at its lowest point. Monthly abundance data were further grouped into seasons for analysis (Table 2.1). No counts were obtained in February 2011 due to the earthquake disruptions in the area. Other missing data were due to poor conditions which made it impossible to get into the water safely to conduct the sampling (e.g. June and September 2011 in Wainui). Sampling was done by way of snorkelling, so the time and sea conditions played a big role in determining when I could get in the water to sample. The quadrat sampling area was from the low tide line to one metre depth. The invasive seaweed *Undaria pinnatifida* was used as a biological indicator for the low tide line as this species is known to be a subtidal species and is found in the three study sites (Russell et al. 2008). The quadrat placements were alternated between the top 50 cm and bottom 50 cm of the sampling area. The lateral distance between quadrats was approximately 1.5 m (Figure 2.3). The position of the first quadrat was random and different every time. The stalk attachment point of the *Pyura* to the substrate determines whether the *Pyura* is inside or outside of the quadrat. The number of *Pyura* inside each quadrat was recorded using an underwater slate.

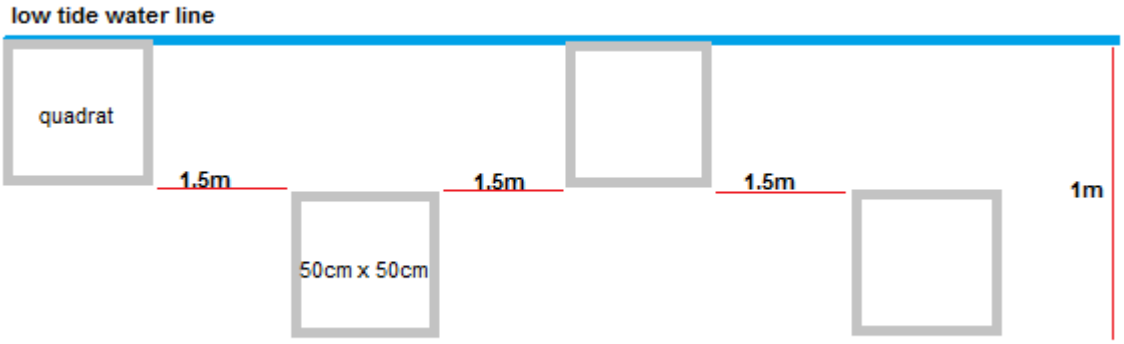


Figure 2.3. Sampling design used to count the abundance of *Pyura pachydermatina* at all sites around the Banks Peninsula. Alternate placement of quadrats was used to work within the top one metre below the low tide water line.

Table 2.1. Months were grouped into seasons as replication for analysis of abundance data.

Months	**Seasons**
January 2011, March 2011, January 2012 - February 2012	Summer
April 2011 - June 2011	Autumn
July 2011 - September 2011	Winter
October 2011 - December 2011	Spring

2.2.3 Size-frequency Data Collection

The length of the tunic of 100 individuals was measured monthly at each of the three study sites. The length measurement was taken from the end of the stalk/start of the tunic to the distal end of the tunic (Figure 1.2). A vernier calliper was used to measure the length of the tunic to the nearest millimetre. The 50 cm x 50 cm quadrat was used to reduce bias towards larger or more visible individuals; using a quadrat helped in focussing the search and monitoring of *Pyura* to specific area. In addition to the range of colours that the *Pyura* tunic can have; the growth of other organisms on the tunic was common (Figure 2.4). These factors could make *Pyura* more cryptic in that the smaller individuals could resemble algal growth on the rocks or larger individuals resembling other macroalgae in the community. The use of

a quadrat was particularly helpful in low water visibility condition in reducing the bias towards larger individuals, and in spotting the smaller or more cryptic *Pyura*.

Figure 2.4. *Pyura pachydermatina* with growth of other organisms on the tunic, in this case mostly algae.

Data collection took place from January 2011 to February 2012. Poor weather or water conditions prevented data collection in a few months at different sites, resulting in missing data for those particular times. In some cases, the water conditions deteriorated while data collection was being carried out. At those times, data collection was stopped and less than 100 individuals were measured (e.g., March and July 2011 in Wainui). A total of 3450 individuals were measured throughout the data collection period. The monthly size data were binned into 10 mm size classes starting from 0-9 mm as the smallest size class and 90-99 mm as the biggest size class. The months were used as replication for season, which is a factor in

the analysis of this dataset (Table 2.1). Further classifications of the class sizes were done for statistical analysis based on gonad analyses (Chapter 4), in which the following groups were created: recruits (< 20 mm), juveniles (20-39 mm), and adults (> 40mm).

All statistical analyses in this section were done using the basic R statistics system (R Development Core Team 2011). A Generalised Linear Model (GLM) was used to analyse the means of abundance and the means of tunic length in the three sites and in the different seasons. GLM was used instead of ANOVA because they were all count data, the distribution of errors was not normal, and the variance was not constant. The quasi-Poisson error term was used for both abundance and size analyses instead of Poisson to compensate for the over-dispersion showed when using Poisson error term (Crawley 2007). The multiple pairwise *t*-test using the Benjamini and Yekutieli method was used as a *post-hoc* test for the GLMs (Crawley 2007). Site, season, and their interaction term (site+season) were the factors and the number of *Pyura* per quadrat (for abundance) and mean size were the responses measured. Size frequency was assessed for each of the sites using the Chi-squared (χ^2) test to investigate if the different life stages of *Pyura* were affected by seasons.

2.3 Results

2.3.1 Preliminary Study

Cumulative means of the number of *Pyura* found inside the quadrats (Figure 2.5) showed that 12 large quadrats or 15 small quadrats would produce a representative mean measure. The large quadrat was thought to be better for abundance counts as this species has a patchy distribution (pers. obs.) which can easily be missed when using the smaller quadrat. The

patchy distribution of *Pyura* meant that the variance of the data was not expected to be low regardless of which quadrat was used. The large quadrat also helped with the practicality and efficiency of sampling when conducting the counting in the water as the larger quadrat was easier to handle and manipulate compared to the smaller one. Less quadrat counts also meant less time would be required for sampling each location.

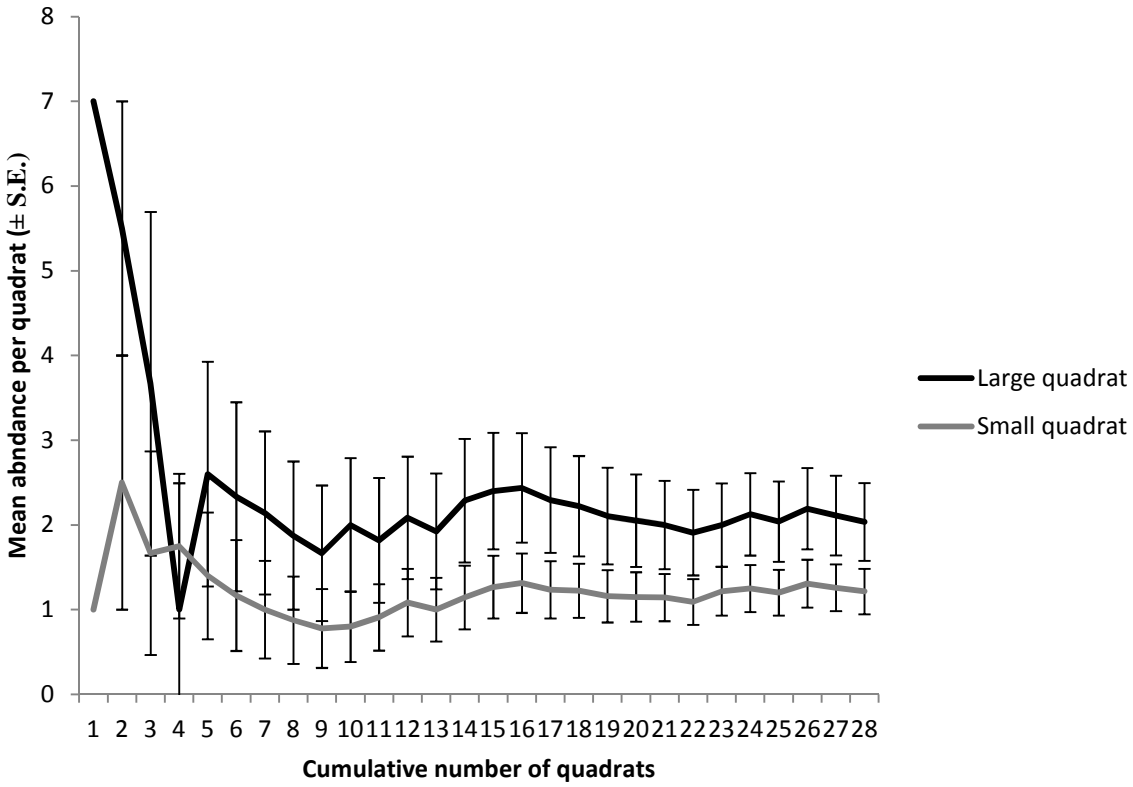


Figure 2.5. Cumulative means of abundance (± Standard Error) of *Pyura pachydermatina* using the large (50 cm x 50 cm) and small (25 cm x 25 cm) quadrats in Camp Bay.

2.3.2 Abundance

The mean abundance for the three study sites showed Pigeon Bay had significantly more *Pyura pachydermatina* with 8.26 individuals per quadrat than Camp Bay with 3.03 individuals per quadrat and Wainui with 1.20 individuals per quadrat (Figure 2.6). The GLM showed that 'site' was a significant factor ($F_{2,404} = 134.264$, $p<0.05$) in determining abundance, while 'season' ($F_{3,401} = 0.163$, $p>0.05$) and 'site+season interaction' ($F_{6,395} =$

0.534, p>0.05) were not significant (Table A1). A model simplification was done to remove 'season' and 'site+season interaction' factors. The 'anova' function in R was used on all the steps in simplifying the model to test for any significant loss of explanatory power. In this case, there was no significant loss of explanatory power in simplifying the model when 'site' alone was left as a factor (Table A2).

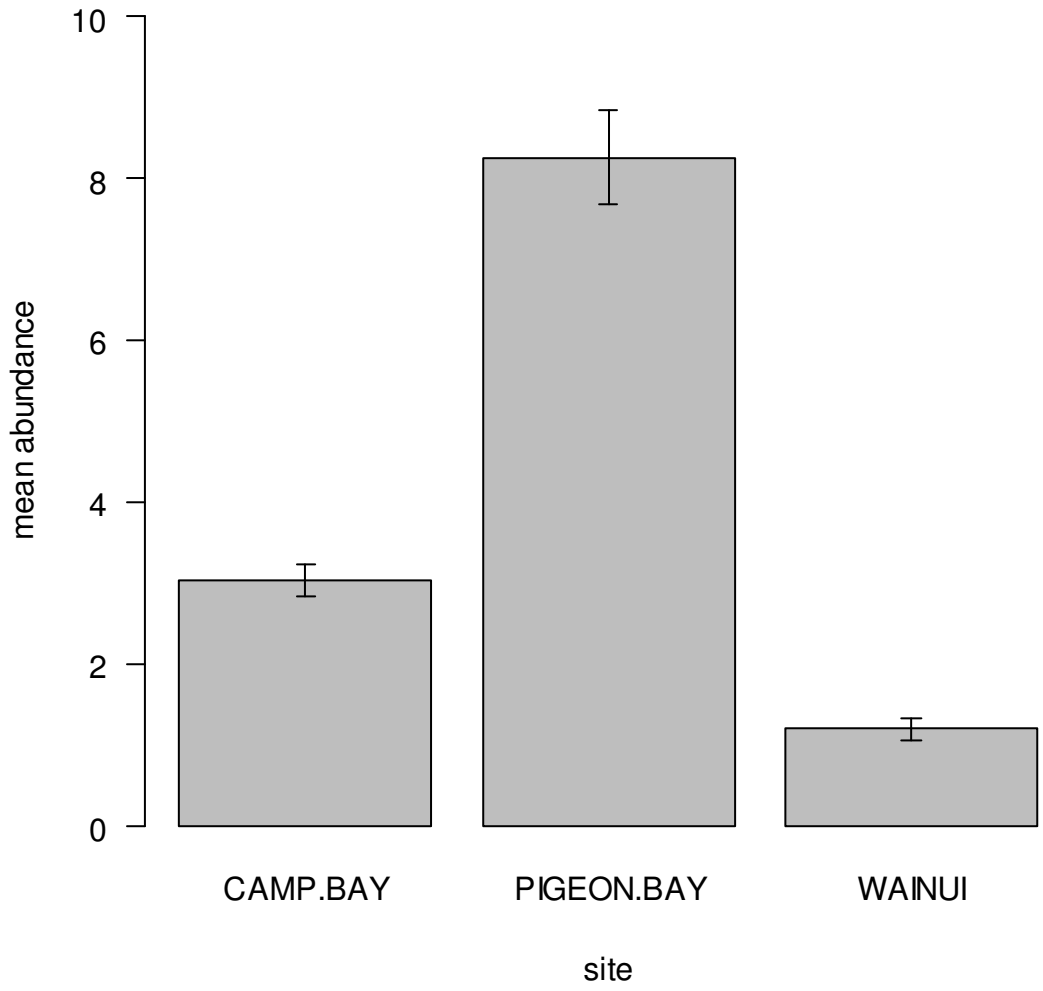


Figure 2.6. Mean abundance of *Pyura pachydermatina* sampled at the three study sites (number per quadrat ± SE) over the period of one year between February 2011 and February 2012.

2.3.3 Size-frequency

The highest mean length of *Pyura* was 61.40 mm observed during winter in Wainui and the lowest mean length was 35.36 mm during autumn in Camp Bay (Figure 2.7). Wainui showed greater variation in the data trend, which may be due to sampling error from weather conditions (Figure 2.8). GLM analysis showed that 'site' was a significant factor ($F_{2,3447}$ = 211.5295, $p<0.05$) in determining the mean size and so were 'season' ($F_{3,3444}$ = 74.3870, $p<0.05$) and 'site+season interaction' ($F_{6,3438}$ = 5.0616, $p<0.05$) (Table A3). In particular, there was a significant difference in the mean size for summer in Camp Bay, autumn in Camp Bay, winter in Wainui, and spring in Wainui (Table A4).

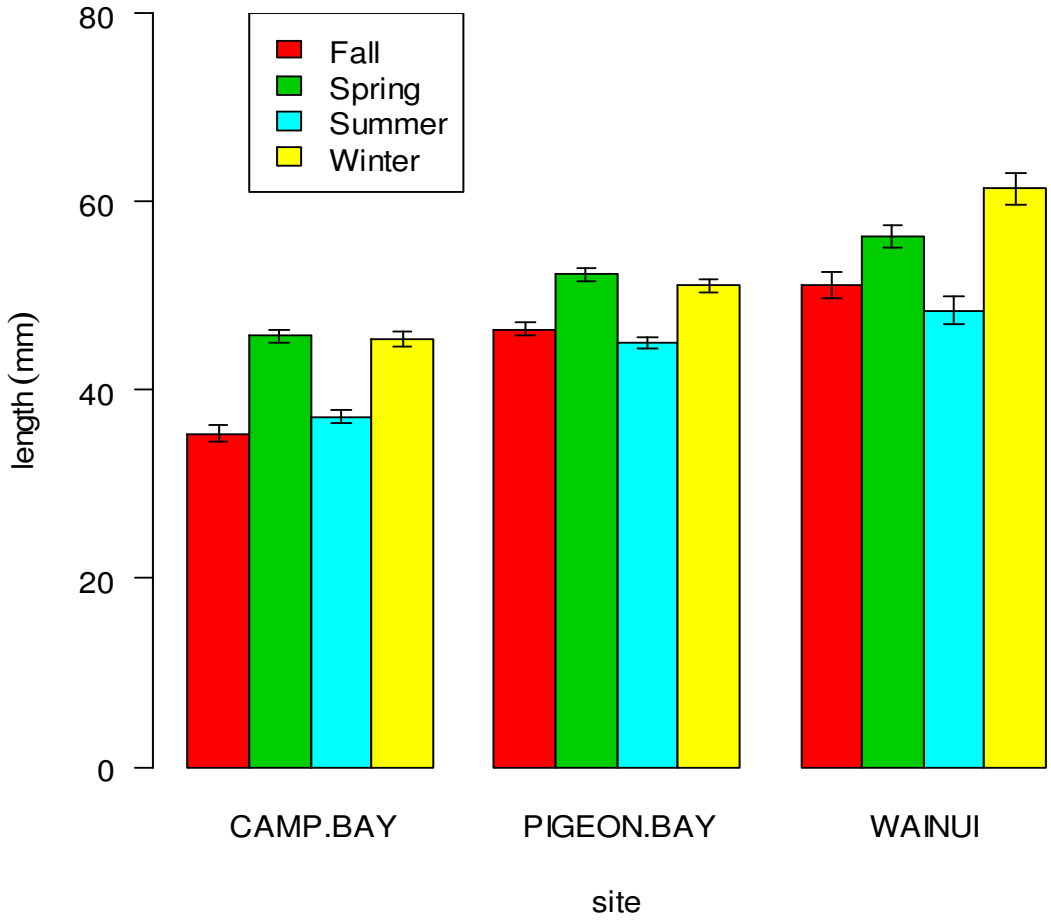


Figure 2.7. Mean length of *Pyura pachydermatina* (± S.E.) in the three study sites over four seasons.

The size frequencies of each site show a range of sizes persisting throughout the year (Figure 2.8), with only the size class under 10 mm not represented in all samples (discussed further in Chapter 4). There was a general increase in mean length of *Pyura* in all three sites over the one year study period.

The frequency of the different *Pyura* life stages (i.e. recruits, juveniles, adults) were affected by seasons in all three study sites (Camp Bay: $\chi^2 = 111.7758$, d.f. = 6, $p < 0.05$; Pigeon Bay: $\chi^2 = 53.6083$, d.f. = 6, $p < 0.5$; Wainui: $\chi^2 = 28.8408$, d.f. = 6, $p < 0.05$). What is interesting was the fact that all life stages of *Pyura* could be found in all seasons at all sites (Figure 2.8).

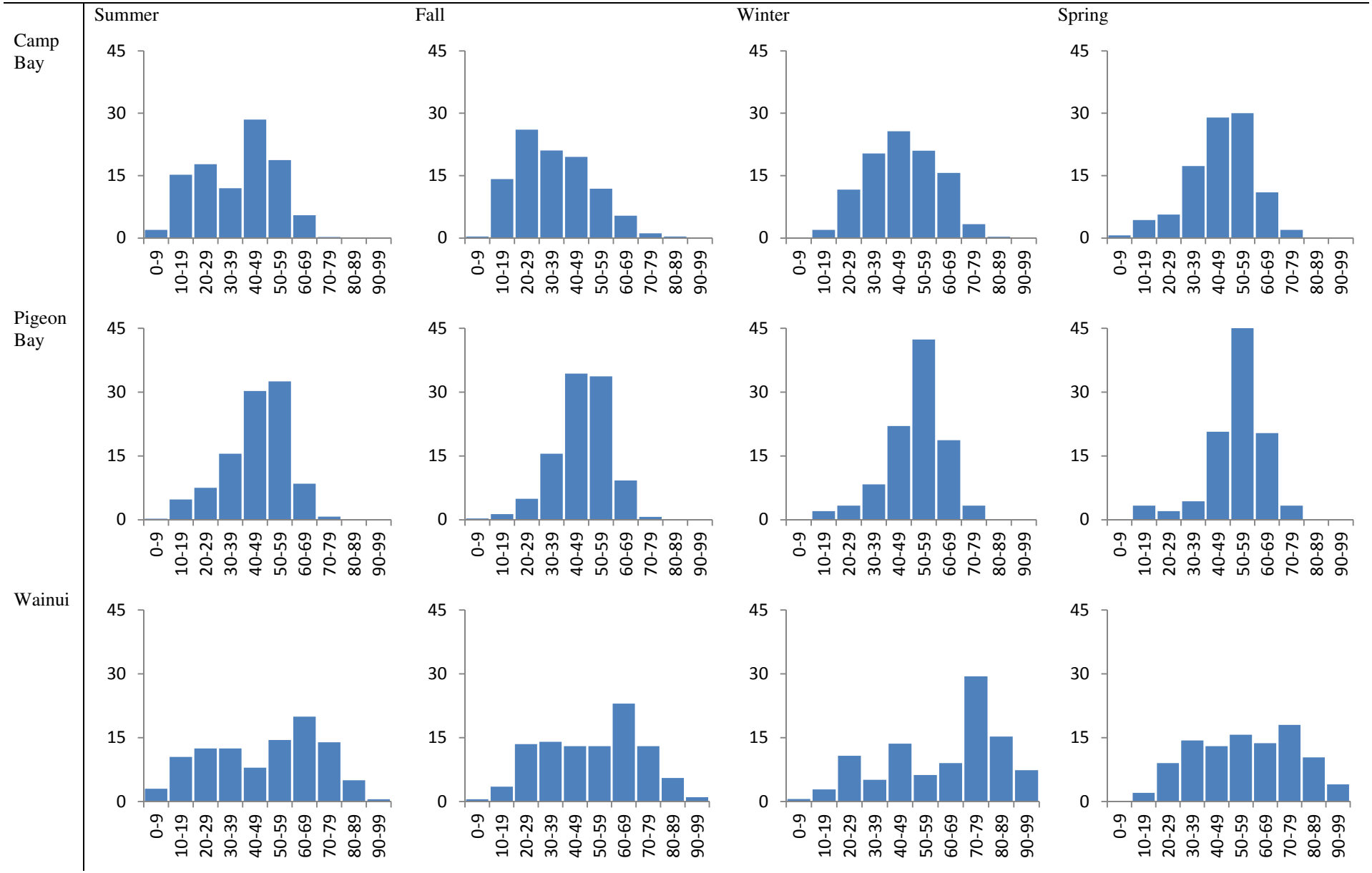


Figure 2.8. Size-frequency of *Pyura pachydermatina* in all three sites over four seasons in ten-millimetre class sizes. The number of *Pyura pachydermatina* is on the y-axis and the size classes are on the x-axis.

2.4 Discussion

The results show that the individuals on the northern sites were significantly different in size and abundance to the southern site. The northern sites both have higher abundances (Camp Bay mean = 3.03/¼ m^2 and Pigeon Bay mean = 8.26//¼ m^2) compared to the southern site (Wainui mean = 1.20//¼ m^2). One other study of this region sampled only the northern side of the peninsula and was conducted over the full subtidal range of the species. My data showed higher abundances compared to the survey data by Schiel and Hickford (2001) where they recorded an abundance of 2-8 individuals per m^2. The marked increase in abundance may be due to the different study sites, Schiel and Hickford (2001) sampled in Godley Head and Taylor's Mistake, or the sampling area where I only sampled the top one metre below the lower tidal line and they sampled the full depth range down to 12 metres-deep. The figures for the mean of abundance can be multiplied by four to get an abundance data per m^2, therefore the adjusted results would be Camp Bay mean = 12.12/m^2, Pigeon Bay mean = 33.04/m^2, and Wainui mean = 4.80/m^2. There was no significant difference in mean abundance of *Pyura* seasonally throughout the year (Table A1).

The different environmental conditions that affect these sites could offer possible explanations for the differences in size and abundance of *Pyura*. Although all the sampling sites are fairly exposed to wave action, they are all facing different directions and as such are affected by different directions of water current. The northern sites are protected from the colder southerly water, whereas Wainui in the southern side of the peninsula is not protected. Unfortunately two of the data loggers deployed in Wainui were lost during the study and water temperature data from the southern side of the peninsula was not obtained and so comparison with the northern side could not be made. These physical environmental

conditions and abiotic factors can contribute to the differences in *Pyura* demography at each of the study sites. The geographical locations of the sites may also contribute to the observed demography of *Pyura* populations around the peninsula. Wainui and Camp Bay are located inside the Akaroa Harbour and Lyttelton Harbour respectively, whereas Pigeon Bay is located outside of the Lyttelton Harbour. The sites located inside the harbours potentially get more protection compared to the site outside which can influence the dispersal of offspring around the area. The studies on the ascidian *Pyura praeputialis* in Chile showed that even though the species can survive outside the bay, the distribution is restricted to only inside the bay (Clarke et al. 1999, Castilla et al. 2004). The authors suggested that the restricted and localised distribution of the species was caused by a specific oceanographic retention mechanism and/or the short larval dispersal period. The same restrictive mechanism could be present in the study sites around Banks Peninsula whereby the populations of *Pyura* are restricted to small areas.

Another possible explanation for these differences could be the ease of access to these sites. The study sites in Camp Bay and Wainui had the easiest access and they were also known recreational areas. Wainui was a popular scuba diving, snorkelling, and outbound destination with regular use by visitors. Camp Bay also had a beach area which is popular with visitors during the summer months; however the study area in Camp Bay is slightly further out from the beach and may not have been approached too frequently. Pigeon Bay was the furthest study site and was only reachable by boat. As such, this site had minimal human impact out of the three which may have contributed to the highest abundance of *Pyura*. Although no observations were made of *Pyura* collection by human, it was observed that the *Pyura* individuals, especially the small ones, higher up on the shore were susceptible to damage by trampling. Further study is required to assess if human use of these rocky shores have any

impact on the populations of *Pyura*. In Camp Bay and Wainui, some shellfish collection was happening throughout the year (pers. obs.) and human activity may have contributed to the difference in mean abundance of *Pyura* in these sites compared to Pigeon Bay. In addition, it was observed that there were more clumps of *Pyura* in Pigeon Bay compared to Camp Bay and Wainui which may also contribute to the high abundance there. It is still not yet understood why some *Pyura* individuals form clumps while others stay solitary, or if there is a preference for clumping or being solitary. A study in Ghana looked at the impacts of human activities in the coastal wetlands and found that they generally have a degrading effect on biodiversity (Wuver & Attuquuayefio 2006). It was also known that exploitation of a habitat can change the balance of biodiversity (Sharpe & Keough 1998, de Boer & Prins 2002).

Size-frequency data gave a snap shot of the population structure over the period of one year (Figure 2.8). χ^2 analyses on the frequency of *Pyura* life stages at each site showed that the life cycle of *Pyura* was dependent on seasons. However, this result may be slightly misleading in that the number of size classes used to make up the three life stages were not balanced, possibly creating some positive bias towards the adult life stage. The presence of all three life stages in all sites at all seasons however, was of great interest to this study. The presence of recruits in every season supports the idea of *Pyura*'s capability for year-round reproduction.

In every season, the largest *Pyura* individuals were found in Wainui compared to Camp Bay and Pigeon Bay (Figure 2.8). Although there was no data on the amount of productivity around the waters of Banks Peninsula, I suggest that the water on the southern side of the peninsula is richer in nutrients than the northern side, leading to the *Pyura* size differences observed. Significant differences in abundance and mean size of *Pyura* in all three study sites

suggest that there were at least three different and separate populations around the Banks Peninsula.

Chapter 3

Reproductive Biology of *Pyura pachydermatina*

3.1 Introduction

3.1.1 Background

Reproduction is one of the most important concepts in biology. It is particularly important to consider when studying the life-history of an organism. Although there are many levels of reproduction such as molecular reproduction and cell reproduction, this study focussed solely on the production of offspring by the adult organism. In the marine realm, there are many reproductive strategies employed by both vertebrates and invertebrates; from broadcast spawning where they release large number of tiny eggs to viviparous species which release live offspring (Andersen et al. 2008). Reproduction in marine invertebrates can be asexual (e.g., cnidarians and sponges) or sexual (e.g., molluscs and gastropods) or both (e.g., colonial ascidians) (Ruppert et al. 2004). However these generalisations are not universal and all of the mentioned examples have exceptions to their reproductive strategies depending on the species. Many may even be able to reproduce both sexually and asexually (e.g., some

cnidarian species). Organisms that reproduce sexually do not necessarily need another individual as many marine invertebrates exhibit hermaphroditism (i.e. possessing both the male and female reproductive organs) (Jarne & Auld 2006).

Generally, tunicates are hermaphroditic but cross-fertilization is typical (Ruppert, Fox, Barnes, 2004); a few species can self-fertilize (e.g., *Ciona savignyi*) (Jiang & Smith 2005). The gonads (testes and ovaries) of tunicates are located either in the gut loop or in the connective tissue of the body wall. Most colonial ascidian species are viviparous and release tadpole larvae (Ruppert et al. 2004) or reproduce by budding (Sugimoto & Nakauchi 1974). *Pyura*, like most solitary ascidians, is oviparous and releases eggs. The eggs, which are typically small with little yolk, are shed from the atrial siphon (Figure 1.2) and fertilisation takes place in the sea (Ruppert et al. 2004, Lambert 2005a). During spawning events, hermaphroditic ascidians can release both eggs and sperm simultaneously. However, some species have been shown to exhibit self-sterility (Rosati & de Santis 1978). Fuke and Numakunai (1996) observed self-sterility of unfertilised eggs in the solitary ascidian *Halocynthia roretzi* and Lambert (2005a) stated that the genus *Pyura* were generally self-sterile. This contradicted the findings of Anderson et al. (1976) who stated *Pyura praeputialis* and *Pyura pachydermatina* were self-fertile. Fertilisation and development of eggs leads to lecithotrophic tadpole larvae which are planktonic and feed off the yolk contained within them. Because the larvae are lecithotrophic, their larval life stage is short. This larval stage can be as short as a few minutes and up to 36 hours long. Before exhausting their yolk supply, the tadpole larvae must find a suitable substratum and attach for settlement and metamorphosis (Ruppert et al. 2004).

The environment plays a major role in ascidian reproduction and development. There are many different types of environmental factors known to affect reproductive and developmental processes in urochordates, of which ascidians are a part. Some of the more commonly studied of these factors include photoperiod (Bingham 1997, Lambert 2005a), temperature (Bourque et al. 2007, Nutsford 2010, Webber 2010), food quality and availability (Lambert 2005b), ocean hydrodynamics (Holloway & Connell 2002), heavy metals (Gallo et al. 2011), and proximity to con-specifics (Bates 2005). The two specific factors this study looked at were light and temperature. Light has been shown to be the primary factor in triggering spawning events in ascidians (Whittingham 1967, Bingham 1997). Changes in ocean temperature are of particular interest as they have been shown to affect gametogenesis and embryonic development, as well as larval recruitment of exotic ascidian species, but not native species (Stachowicz et al. 2002).

3.1.2 *Pyura* reproduction

As a broadcast spawner, *Pyura pachydermatina* releases a large number of eggs during spawning. The strategy of producing a large number of small eggs has been demonstrated to be optimal in patchy environments such as those in which *Pyura* is found (Winemiller & Rose 1993). In novel environments, adults might initially be isolated or scattered and less able to find a mate. The viability of self-fertilisation in *Pyura pachydermatina* has been tested by Anderson *et al*. (Anderson et al. 1976), but this earlier study was contradicted by Lambert (2005a) which stated that Pyurids generally were self-sterile. Other observational studies of some *Pyura* species (e.g., *Pyura stolonifera* and *P. praeputialis*) suggested that individuals released eggs and sperm at slack tide, intermingled in a viscous matrix on top of spawning individuals, thus increasing the potential for self-fertilisation (Marshall 2002, Castilla et al.

2007). Preliminary attempts to reproduce *P. pachydermatina* in the laboratory over the summer of 2009/2010 showed that this species was readily induced to release gametes indicating that reproductive experiments were viable (Goldstien & Bleyendaal unpublished data).

3.1.3 Chapter objectives

This chapter focussed on the reproduction of *Pyura pachydermatina*. The potential for self-fertilisation (or selfing) in *P. pachydermatina* was tested by carrying out spawning experiments in the laboratory. The questions that this chapter investigated were:

- Is *Pyura pachydermatina* capable of selfing/self-fertilisation?
- Is there any significant difference in the reproductive potential of isolated individuals compared to those in the presence of con-specifics?

It was very important to confirm the potential of *Pyura* to self-fertilise after two contradicting findings (Anderson et al. 1976, Lambert 2005a), as this characteristic could be pivotal towards the species survival and persistence in novel environments such as harbours, wharfs, and marinas.

Coincidently, these novel environments are usually the entry point for non-indigenous species which could lead to increased competition for resources and therefore increased pressure for reproductive success. A study in Maine, USA, looked at the difference between four artificial and four natural substrates for colonisation and growth of native and exotic fouling species. The natural substrates showed little change in the abundance of both native and exotic

species, but the artificial substrates showed drastic increase in abundance of exotic species and decline in native species (Tyrrell & Byers 2007). In such case, the reproductive strategy of the native species must be giving it an advantage over the exotic species for successful establishment. This aspect of invasive pressure by NIS and invasion resistance by the native species is discussed in more depth in Chapter 5.

3.2 Methods

Spawning experiments of *Pyura pachydermatina* were carried out from January 2011 to February 2012. This was conducted every six weeks in a temperature controlled laboratory. Due to the earthquake and problems with the room temperature controls, the experiment was disrupted between February 2011 and May 2011.

15 individual *Pyura* were collected every six weeks from Camp Bay (away from abundance monitoring site of Chapter 2). When the weather or water condition was not favourable, collections were done from the floating pontoon in Magazine Bay (Figure 2.2). A minimum tunic length of 40 mm was selected as the criteria for individuals collected for the spawning experiments. The specimens were then transported back to the laboratory within two hours of collection in a zip-lock bag placed in an ice container. The specimens were then placed in filtered sea water for up to three hours to let them filter out their gut content which passed out as faeces. This allowed for a cleaner experiment which greatly helped when counting the eggs or larvae.

The specimens were divided into two treatments differing in the number of individuals per treatment. One treatment had a single *Pyura* and the other treatment had multiple (four) individuals. The treatments were designated as ‘single’ and ‘multiple’ respectively. The

experiment was carried out in 40 x 30 x 20 cm plastic containers filled with filtered sea water with three replicates of each treatment. The animals were tied to a piece of string on their stalk and were suspended mid-water inside the containers (Figure 3.1). Temperature data loggers were placed suspended inside the containers to ensure the temperature of the water matched that of the natural environment during the different seasons. The mean monthly water temperature (Figure 1.4) was used as the reference temperature and the room's temperature setting was adjusted accordingly. Aquarium air bubblers were placed in each of the containers to aerate the water throughout the experiment.

Spawning was triggered by way of manipulating light and darkness. A light regime of 12 hours light and 12 hours dark was used to induce *Pyura* to spawn. The experiment was left to run for one week and observations were carried out at day one, three, and seven after set up. Red lights were used in the laboratory only when observations were made during the simulated dark periods (Figure 3.2).

When eggs were observed, the contents of the container were sieved through a 63 µm mesh to collect all the eggs and larvae that were present. The container was then refilled with filtered sea water and the experiment continued until the maximum time of one week. The eggs and larvae collected were then placed in a 50 ml plastic beaker filled with filtered sea water. A serial 1:10 dilution was carried out when necessary so that counting of eggs and larvae was viable. Three millilitres subsamples were pipetted out into a Petri dish and filtered sea water was added before the eggs and larvae were counted under a stereo microscope. The total counts of eggs and larvae from each container were recorded for the duration of the experimental week. Total counts of eggs and larvae less than 100 was treated as spill-over contamination and counted as nil, because there was only one sieve available for use during

the experiment. A generalised linear model (GLM) on the total eggs and larvae count between the two treatments was performed in R (R Development Core Team 2011).

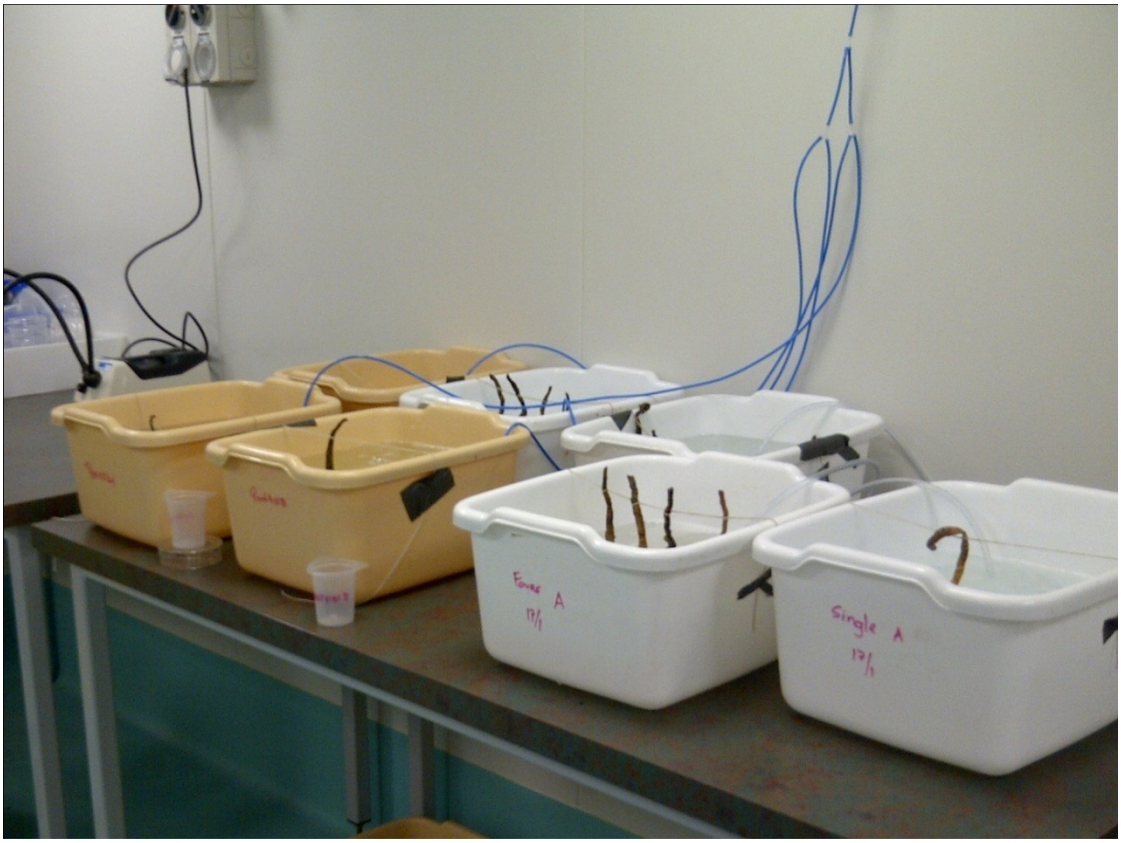


Figure 3.1. Spawning experimental set up in the laboratory with *Pyura* individuals tied to strings and suspended mid-water in plastic containers.

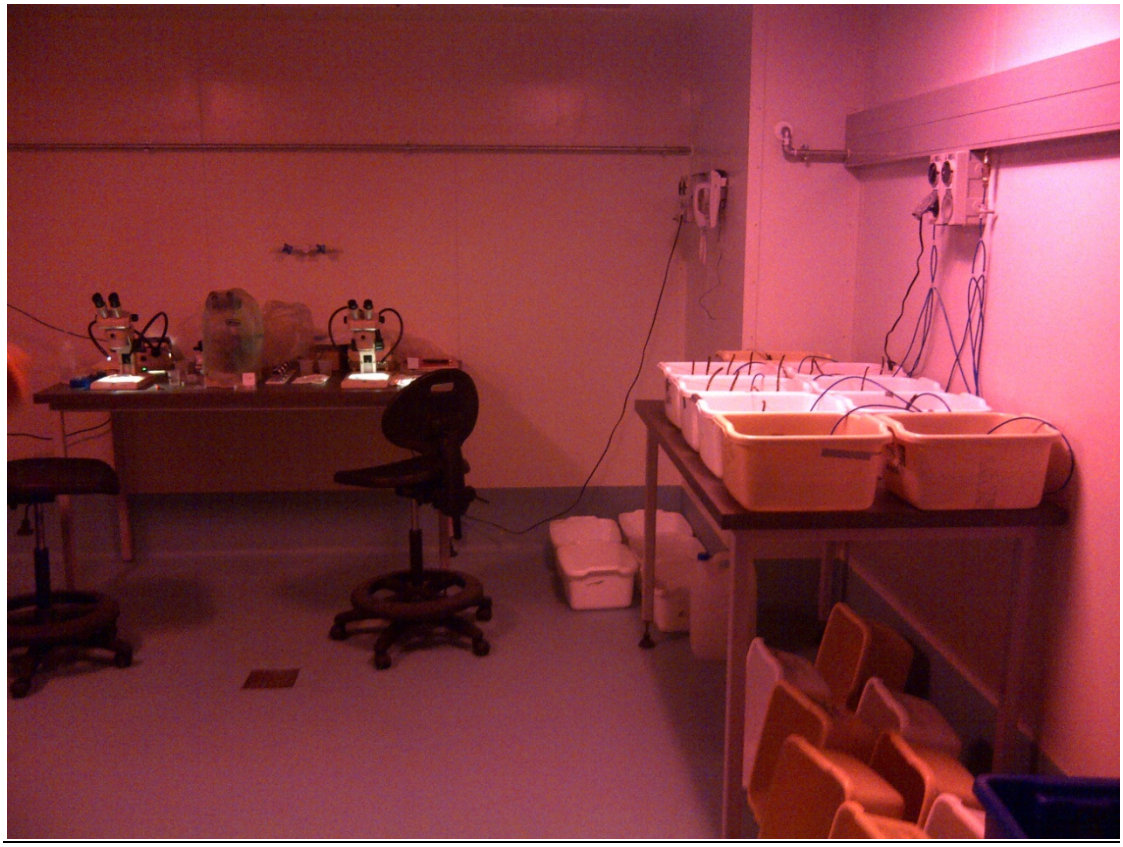


Figure 3.2. Laboratory set up with red lights used when observing for spawning in simulated darkness.

3.3 Results

Any spawning that occurred one day after the start of the experiment resulted in only eggs being present in the containers. Larvae were always present when the *Pyura* have spawned and were usually observed on the third or seventh day after the experiment was set up. This indicated that the offspring were successfully fertilized and were viable. Eggs and larvae were microscopic in size. The eggs were generally ~250-300 µm in diameter and the larvae were ~900 µm in length (Figures 3.3 and 3.4).

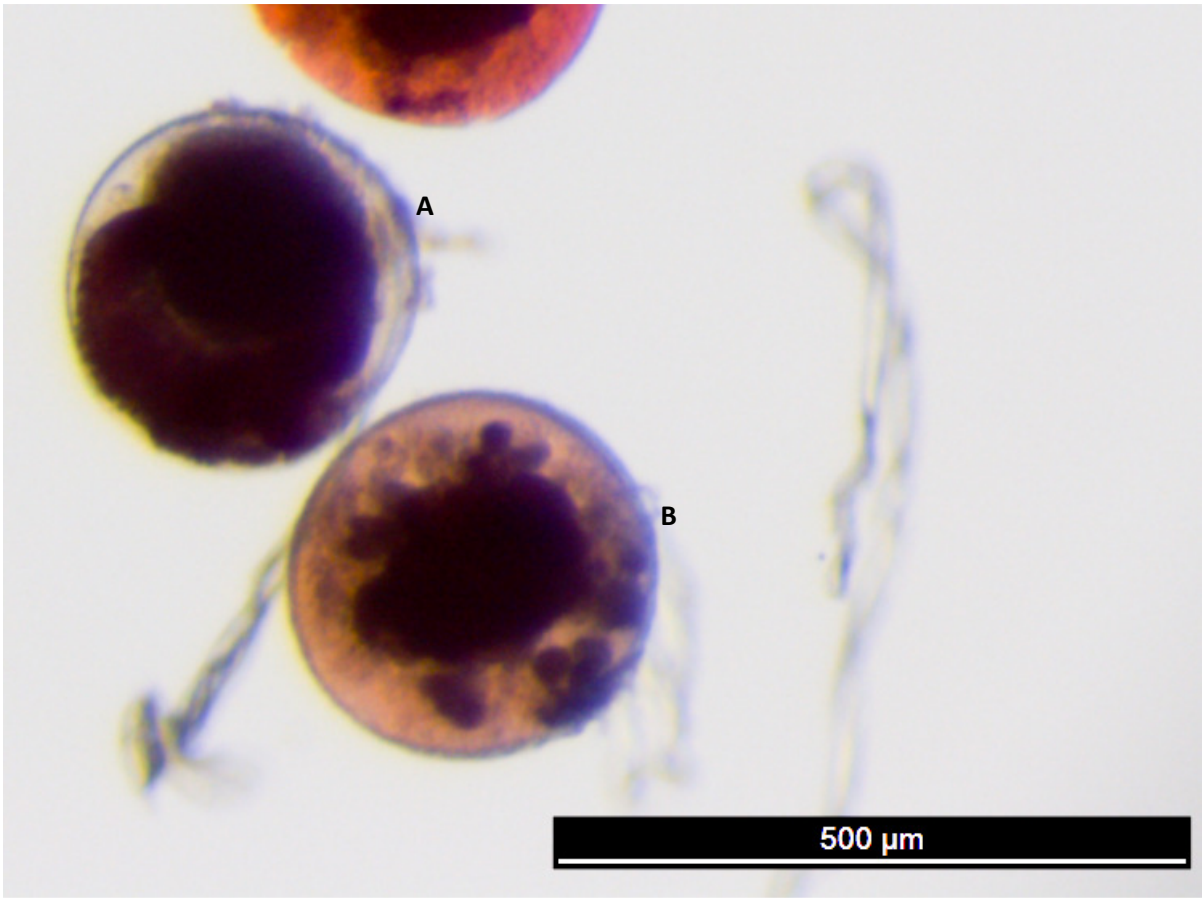


Figure 3.3. Eggs of *Pyura pachydermatina* viewed under a stereo microscope. Egg **A** on the left has started to develop a tail encircling the embryo inside. Tail formation usually starts five hours post-spawning and completes in about ten hours. Egg **B** on the bottom was younger with smaller embryo and no signs of tail development yet.

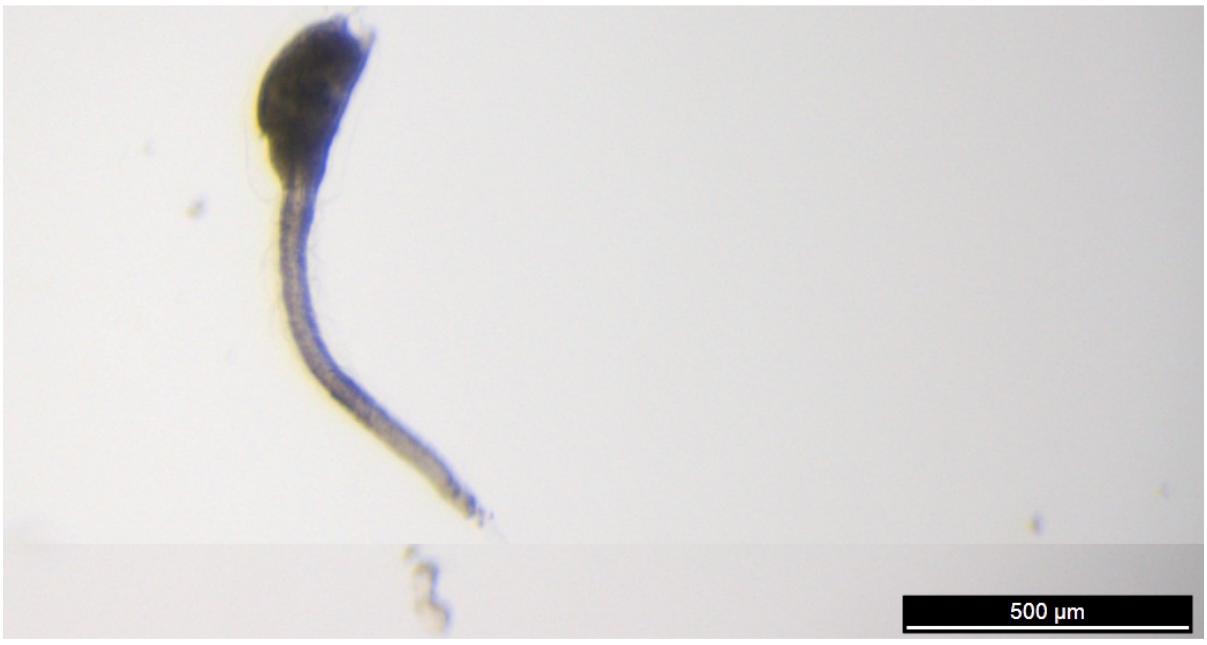


Figure 3.4. Larva of *Pyura pachydermatina*. Hatchings of larvae begin at 12 hours post-spawning. The anterior papillae in *Pyura pachydermatina* larvae are prominent and are used for attachment and settlement.

In the ‘single’ treatment, in seven of nine spawning trials; at least one out of the three replicates spawned successfully (Figure 3.5). This showed that a single isolated individual *Pyura* can spawn and produce viable offspring on its own, thus confirming selfing capability. In the ‘multiple’ treatment, spawning was successfully observed in at least two out of the three replicates throughout the year (Figure 3.5). Spawning frequency was highest in December 2011 when the entire ‘single’ and ‘multiple’ treatment replicates successfully spawned and lowest in June 2011 when only two out of the three ‘multiple’ treatment replicates managed to spawn (Figure 3.5).

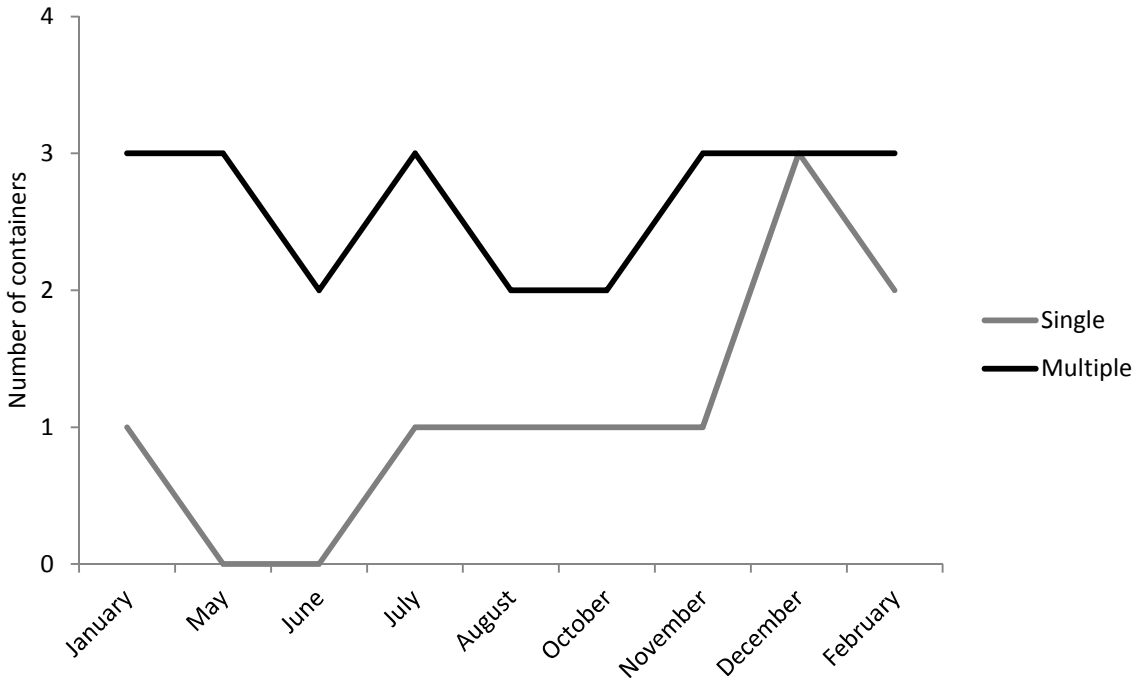


Figure 3.5. Number of containers/replicates where eggs and/or larvae were observed from January 2011 to February 2012.

The mean total output (eggs and larvae) from the ‘single’ and ‘multiple’ treatments was 60552.4 and 130759.3 respectively (Figure 3.6). A GLM with quasipoisson error distribution showed that the number of individuals in the treatments (i.e. one vs. four) had a significant impact in the mean number of total output ($F_{1,32} = 4.6346$, $p<0.05$). This significant difference

was expected as more individuals were expected to give higher number of offspring when compared to single individuals. However, the difference of mean total output was only by two-fold (~2.16) when the number of individuals in the treatments differed by four-fold.

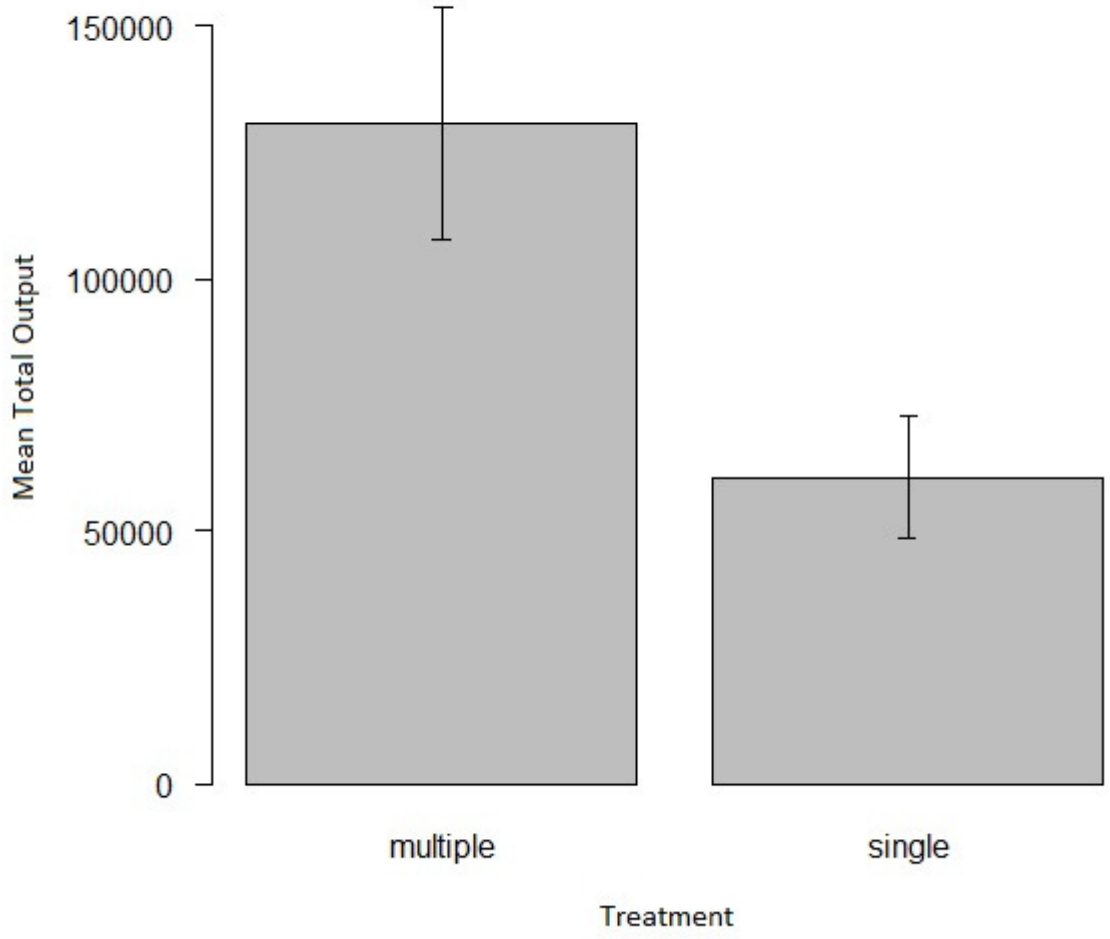


Figure 3.6. Mean total outputs of eggs and larvae (± S.E.) from the two treatments from all successful spawning experiments.

3.4 Discussion

Spawning occurred in a total of ten containers of the 'single' treatment and a total of 22 containers of the 'multiple' treatment throughout the year (Figure 3.5), as the frequency of spawning was higher in the 'multiple' treatment compared to the 'single' treatment. Nonetheless, some of the isolated individuals released eggs and fertilised them to produce viable larvae. The results from the 'single' treatment experiments confirmed the self-fertilisation capability of *Pyura pachydermatina* and supported the findings of Anderson et al. (1976). Several studies have been carried out on other marine invertebrates to test for self-fertilisation (Jiang & Smith 2005, Manríquez & Castilla 2005, Johnson 2010). It was suggested that self-fertilisation could be a potential insurance for the species against adverse conditions for cross-fertilisation (Ryland & Bishop 1990, Manríquez & Castilla 2005). An example would be a natural patchy distribution of a species which might isolate an individual far enough to prevent cross-fertilisation, as might be the case with *Pyura pachydermatina*. Self-fertilisation also reduced the risks associated with sex, including finding an appropriate mate in copulating species or dilution of gametes in spawning species (Lively & Lloyd 1990, Johnson 2010).

The *Pyura* specimens selected for this spawning experiment had a minimum tunic length of 40 mm. Histology of the gonad tissues showed that it was the minimum size of *Pyura* that have developed its male and female gonads to maturation (more details in Chapter 4). Although we tried to collect specimens of similar size, a maximum size limit was not set for this experiment. In some species, size has been directly linked to fecundity whereby larger individuals produced higher numbers of offspring and vice versa (Hislop 1988, Honěk 1993). The individuals used for the spawning experiment were placed in their containers at random to eliminate bias due to size variation.

The evolution of selfing was thought to be dependent on the trade-offs of the various costs and benefits associated with it (Manríquez & Castilla 2005, Johnson 2010). Ryland and Bishop (1990) stated that selfing was the most extreme form of inbreeding that reduced the level of heterozygosity to almost zero. Probably the most common argument against selfing is that of inbreeding depression. This might lead to decreased survival, growth rate, or fecundity of the species due to retention of recessive deleterious alleles (Charlesworth & Charlesworth 1987, Johnson 2010). This in turn led to the suggestion for the mechanisms of self-sterility in some species to prevent inbreeding depression (Jiang & Smith 2005). Inbreeding depression, however, was not ubiquitous in sessile organisms (Cheptou et al. 2000, Culley 2000, Manríquez & Castilla 2005). It has been suggested that for species with limited dispersal potential, inbreeding might be more prevalent. For these species, this could lead to increased tolerance for inbreeding, culminating in populations that were able to self with little or no inbreeding depression (Johnson 2010). It was also suggested that if maintained over a number of generations, selfing species would tend to purge deleterious recessive alleles from the population, thus reducing inbreeding depression (Ryland & Bishop 1990). In this study, even though selfing capability of *Pyura pachydermatina* was confirmed, the fitness of the offspring was not tested. Generally, studies on selfing in marine invertebrates are carried out under laboratory conditions and there has been a lack of experimental approaches in the field. Therefore, the likelihood or frequency of selfing occurring in the wild is not well documented (Jiang & Smith 2005).

Synchronous spawning was thought to be critical for marine invertebrates that release gametes into the water column and rely on water currents for fertilisation (Manriquez & Castilla 2010). Many environmental factors such as water temperature, light, and presence of

con-specifics might act as cues for populations to spawn in synchrony and maximise fertilisation success. In order for these environmental cues to effectively set off synchronous spawning, they must be distinct enough for the organism to sense and must be repeated reliably to be used year after year (Bingham 1997). In the natural environment, day-length is highly predictable and does not show much interannual variation. It could be regarded as one of the most dependable gametogenic cues, hence, the use of light and dark periods in this experiment. All spawning events that were observed during the experimental period occurred in darkness. First signs of spawning usually took place between two and five hours after dark. Not all the gametes were released at once when spawning took place. Rather, they were released in pulses. There seemed to be no pattern to the timing or amount of gametes released by the pulses, although only visual observations were made. The thick and opaque tunic of *Pyura* would not have let much light through, therefore it was unlikely that light was the only factor affecting its spawning periods. This was a contrast to *Chelyosoma productum* which has a thin translucent tunic through which light could pass easily and lead to its gametogenesis being heavily influenced by light (Bingham 1997).

Temperature of the water in the experimental containers varied depending on the mean sea temperature at the time at which the experiment was carried out. The lowest temperature was ~8°C recorded in August 2011 and the highest was ~18°C in January 2012 (Figure 1.4). There was no observable pattern between water temperature and spawning capability in *Pyura* from this study. This suggested that temperature probably played a very minor role, if at all, as a gametogenic cue for this species.

The number of gametes produced throughout the spawning experiments varied greatly. The lowest number of total output (i.e. eggs and larvae) counted was just over 1500 in June 2011

from one of the ‘multiple’ treatment replicates. The highest number of eggs and larvae counted from one container was over 369000 in November 2011, also from a ‘multiple’ treatment container. It was expected that the ‘multiple’ treatment containers would yield higher total output when compared to the ‘single’ treatment containers simply from the fact that they had more *Pyura* individuals. However, the difference in mean of total output between the two treatments was only about double in the ‘multiple’ compared to the ‘single’ treatments (~2.16) (Figure 3.6). Interestingly, the number of replicates that successfully spawned in the ‘multiple’ treatment was also about twice as many as those in ‘single’ treatment (22/10 = 2.2). It was not known how many individuals from the ‘multiple’ treatment actually released offspring when spawning was observed. Further study is required to find out if the presence of con-specifics had an effect on the number of individual *Pyura*’s spawning in synchronicity.

Pyura pachydermatina’s distribution in the man-made structures of the Port of Lyttelton is patchy and some individuals have been found to be very isolated (pers. obs.). The Port of Lyttelton was also the entry point for the invasive ascidian, *Styela clava* (Webber 2010). Selfing by isolated *Pyura*’s in these novel structures might increase their resilience towards possible invasion from *Styela clava*.

Chapter 4

Reproductive Pattern of *Pyura pachydermatina*

4.1 Introduction

4.1.1 Temporal variation in reproduction

Marine organisms display a wide range of reproductive strategies within and among species. These strategies include, hermaphroditism, broadcast spawning, and brooding of young, as well as temporal variations driven by environmental change or seasonal patterns (e.g., light, temperature, resource availability) (Bates 2005, Lambert 2005a, Johnson 2010, Smart et al. 2012). For example, sessile invertebrates of the shallow water, including those in the intertidal zone, are constantly exposed to regular abiotic environmental changes. For many of these species which include the ascidians, the response towards these regular changes is to have a reproductive pattern that follows the seasonal cycle (Hirose et al. 2005, Bourque et al. 2007, Fukuda & Hirose 2008). Bingham (1997) outlined the basic criteria these abiotic environmental factors must fulfil in order to have an impact on the reproductive patterns of the marine organisms. The criteria included:

- biologically relevant to the organism.
- reliable and on repeated cycle.
- gradually build to allow for the organisms to develop their gonads before spawning.

Although a lot of these environmental factors have been studied as cues that trigger spawning events, the sensitivity of the organisms and the degree of response towards these factors varies a great deal among species (Bingham 1997, Bates 2005, Bourque et al. 2007). For example, gametogenesis in the ascidians *Boltenia villosa* and *Styela gibbsii* was unaffected by a reversal in their light regime, but gametogenesis cycle in *Chelyosoma productum* was drastically changed (Bingham 1997). Water temperature was a factor that has been repeatedly studied and is known to affect the reproduction pattern of many marine invertebrates (Bingham 1997, Parker et al. 1999, Ren et al. 2003). For instance, the ascidian *Ciona intestinalis* is greatly affected by temperature in terms of their maximum age, growth, and reproduction. When water temperature is colder, the age of *C. intestinalis* increases, growth rate decreases, and reproduction rate also decreases. The opposite to these trends is true for *C. intestinalis* in warmer water (Dybern 1965).

There have been studies that show distribution of marine invertebrates play a role in their reproductive adaptation depending on the local climate. For example, the invasive ascidian *Styela clava* has a summer reproduction period and highest recorded reproduction was in August for the species in Canada during the boreal summer (Bourque et al. 2007) and in December for the same species in New Zealand during the austral summer (McClary et al. 2008). The marine environment or ecosystem can be divided into three main climate zones which include the tropical zone, the temperate zone, and the polar zone. The water temperature and day-length in the tropics show minimal variation throughout the year. In contrast, those two environmental factors in the temperate zones can fluctuate greatly

between seasons. The polar zones have minimal variation in water temperature throughout the year, but changes in day-length can vary greatly, albeit on a much longer time scale than the seasonal changes in temperate zones. Studies on marine invertebrates have shown some generalities regarding reproductive patterns in those three climate zones (Rokop 1974, Bauer 1992). For example, year round reproduction could be observed mainly in the tropical zone for the soft coral species *Dendronephthya hemprichi* and the Antarctic ascidian *Cnemidocarpa verrucosa* in polar habitats (Dahan & Benayahu 1997, Sahade et al. 2004). The colonial ascidian *Tridemnum solidum* is another example of an invertebrate species that inhabits the tropical zone and reproduces all year-round (van Duyl et al. 1981). In contrast, seasonal reproduction was mainly observed in the temperate species such as the ascidians *Diplosoma virens* and *Didemnum molle* occupying the rocky shores habitat in the temperate zones of Japan (Hirose et al. 2005, Fukuda & Hirose 2008). Studies on *Halocynthia hilgendorfi ritteri* (Choi et al. 2004) and *Styela clava* (Parker et al. 1999) are some examples of temperate ascidians that exhibit seasonal reproduction. However, these generalisations on the effect of variations in water temperature and light on reproduction were not absolute and there have been some exceptions to them (Lambert 1968, Sahade et al. 2004). The reproductive patterns in different climate zones were also not limited to species. Populations of the same species distributed in different climate zones have been shown to exhibit different reproductive patterns. For example, the solitary ascidian *Herdmania momus* in the Mediterranean has a seasonal reproduction period, whereas the individuals in the Red Sea exhibit year-round reproduction (Shenkar & Loya 2008).

Pyura pachydermatina as a temperate ascidian has been shown to have a winter spawning period (Anderson et al. 1976, Egan 1984b). In contrast to these findings, Goldstien and Bleyendaal have found the presence of larvae in the water column during the summer months

of 2009 and attempts to induce spawning during the same months were successful (unpublished data). Their findings suggested the potential of a year-round reproduction period for *Pyura*. Hence one of the main focuses of this study was to establish the temporal pattern of reproduction for this species. Year-round reproductive pattern for a temperate species was an intriguing phenomenon as it would be different from many other temperate marine invertebrates that reproduce seasonally.

4.1.2 Year-round reproduction: advantages

Many of the temperate marine invertebrates reproduced seasonally during summer months associated with an increase in water temperature (Pearse 1978, Lista et al. 2006, Rius et al. 2009, Smart et al. 2012). Winter reproduction, as has been reported for *Pyura pachydermatina* (Anderson et al. 1976, Egan 1984b), can leave a species vulnerable to competitive exclusion from those species that reproduce during the summer period. For example, the offspring of summer-reproducing ascidians such as *Styela clava* or *Ciona intestinalis* would have an advantage in using up resources (e.g., space) when there is less competition from offspring of winter-reproducing species (Ramsay et al. 2008). This negative effect could be further exacerbated by the die-off of adults during the late spring to early summer, as is the case for *Pyura pachydermatina* (Egan 1984b), when other species were spawning and colonising the vacated spaces. Conversely, it could be argued that the asynchrony of seasonal reproduction might benefit those reproducing in winter period as there would be less competition for resources for the offspring.

Year-round reproduction would allow the constant input of offspring into the environment, giving them a higher chance of finding favourable habitats (Dahan & Benayahu 1997).

Reduced competition for suitable settlement space could be a major benefit for the species, especially in novel areas such as man-made structures which have been suggested to have the potential to increase the abundance and diversity of subtidal epibiota (Connell & Glasby 1999). Thus, year-round reproduction and less competition in novel areas can potentially increase a species' resistance to invasive species with a seasonal reproductive period.

4.1.3 Chapter objectives

Contrasting evidence questions the winter reproduction period of *Pyura pachydermatina*. This chapter investigates the reproduction pattern of *P. pachydermatina* around Banks Peninsula on the South Island of New Zealand. The overriding question to be answered was:

- Does *Pyura pachydermatina* exhibit a year-round reproductive pattern?

Several aspects of the early life history of the species were tested; from gonad indices and spawning periods to larval recruitment. Other questions to be answered in this chapter were:

- Is there temporal variation in the reproductive output of *Pyura pachydermatina*?
- Are larvae/recruits present in the water column all year-round?
- Is gonad tissue in this species proportional to body size?
- Is gonad development in this species affected by seasons?

4.2 Methods

4.2.1 Spawning experiment

The laboratory spawning experiment outlined in Chapter 3 was grouped into seasons to investigate the seasonal variation in *Pyura*'s reproductive potential (Table 4.1).

Table 4.1. Seasonal grouping of spawning experiments conducted between January 2011 and February 2012.

Month	Season
January 2011, February 2012	Summer
May, June 2011	Autumn
July, August 2011	Winter
October, November, December 2011	Spring

4.2.2 Recruitment in natural environments

Four recruitment-rigs were put together to capture larvae of *P. pachydermatina* in the natural environment between January 2011 and February 2012. Each rig consisted of three grey-coloured 15 x 15 cm PVC settlement plates that were attached to a white PVC pipe (Figure 4.1). These plates were preconditioned by immersion in sea water for a minimum of two days before they were used. Goldstien and Bleyendaal reported that *Pyura* larvae could successfully settle on these plates (unpublished data). Ropes, anchor-weights, and buoys were used to keep the rigs in place. The PVC settlement plates were designed to "hang" face down at 5 metres below the surface of the water (Figure 4.2). These rigs were deployed at four sites. Two were placed in the east and west sides of Pigeon Bay, and the other two were anchored in the east and west sides of Camp Bay (Figure 2.2). The maximum distance between the rigs and the rocky coast was approximately 10-12 metres. The settlement plates were left out in the water for one month after which they were replaced with clean plates. When conditions were poor and it was impossible to get to the plates safely, they were left for an extra month before being replaced. Settlement plates were collected and placed in plastic bags and kept in an ice container during transport to the laboratory. They were then immersed in sea water and observed under a stereo microscope for the presence of *Pyura* settlers.

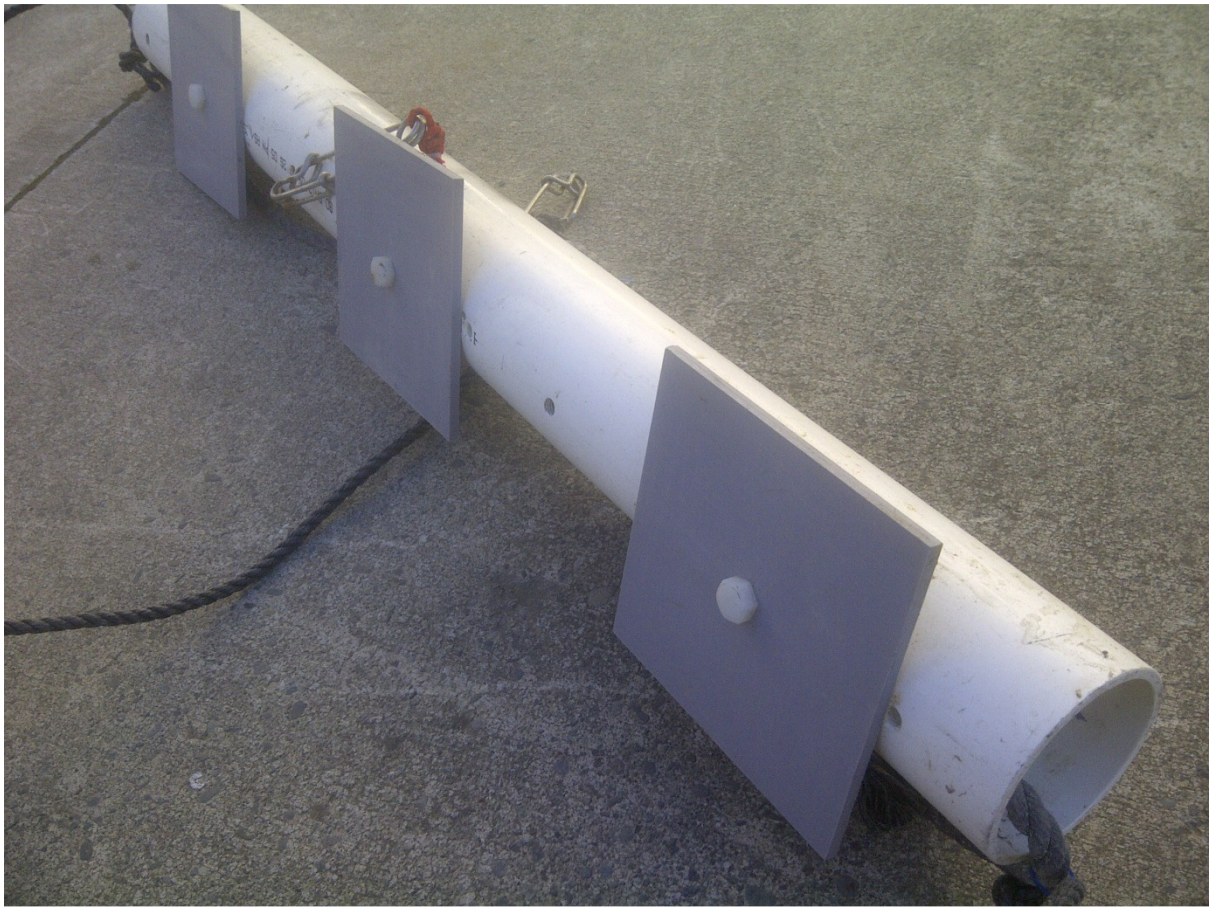


Figure 4.1. Design of the settlement-plate rig used in an attempt to capture *Pyura pachydermatina* larvae from the water column.

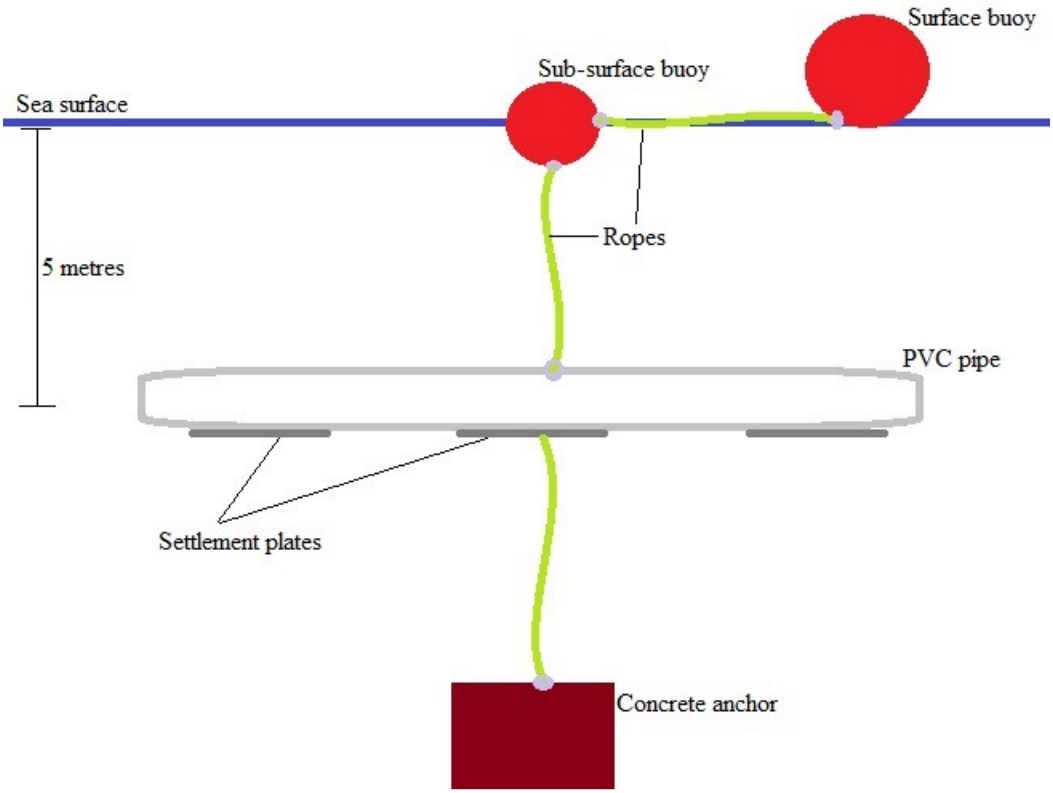


Figure 4.2. Simplified design of the settlement plate rig in the water, showing the downward facing settlement plates and the ropes, buoys, and anchor keeping it in position.

4.2.3 Gonad indices

Nine collections of five adult *Pyura* individuals were made between May 2011 and February 2012 for study of their gonads. At the time of collection, an individual with a minimum tunic length of 50 mm was considered to be adult. An additional 20 individuals of varying sizes were also collected in May and June to study the gonad maturation stage in relation to body size. All the weights were wet weights obtained during dissection. 'Total body weight' (TBW) included the weight of the tunic and the weight of residual water trapped within it when the animal was removed from water. However, TBW did not include the weight of the stalk which was cut off prior to dissection. 'Visceral mass' (VM) was the weight of the animal enclosed within the tunic. VM was obtained by cutting and removing the tunic, making a small incision to allow drainage of water, blotting it dry, and then weighing the

"fleshy" tissue (Figure 4.3a). Further dissection of the visceral mass revealed two rows of gonad tissues on either side of the body. Each row was arranged into lobes which contained both the male testes and female ovaries (Figure 4.3b). The two rows of gonad tissues were removed and weighed for every specimen collected (GW). These weight measurements were then used to calculate gonad indices, which were subsequently used in correlation analyses between the different weight measurements and body size (Equation 4.1 and 4.2).

Equation 4.1: $$\textbf{\textit{Gonad index 1}} = \frac{\textit{gonad weight}}{\textit{visceral mass}} \times 100$$

Equation 4.2: $$\textbf{\textit{Gonad index 2}} = \frac{\textit{gonad weight}}{\textit{total body weight}} \times 100$$

One lobe of the gonads was then dissected off and placed in a 10% formalin-seawater solution. Fixing of gonad tissues in the formalin solution lasted for a minimum of 48 hours. After fixation, the gonad tissues were rinsed in water and dehydrated in an alcohol series according to Figure A1. Dehydrated samples were then embedded in paraffin wax and blocked. The blocks were cut to a thickness of 7µm with a microtome, and stained with haematoxylin and eosin, according to standard staining procedures (Howard & Smith 1983).

Histological slides of *Pyura* gonads were examined and the maturation stage of the testes and ovaries were classified into one of the five stages outlined in Table A5.

R-squared values of gonad indices 1 and 2 (Equations 4.1 and 4.2) were calculated to find the weight that most accurately represented the growth of the animal. Fisher's Exact tests were performed in R (R Development Core Team 2011) on both the ovaries and testes to test for dependency between the gonad developmental stages and season. The effects of seasons on the male and female mature gonads was also examined by a Chi-squared (χ^2) test. Only

gonads in stage 4 development were used for the χ^2 test, because I wanted to investigate if seasons have an effect on the simultaneous presence of mature ovaries and testes (Table A5). The monthly mean gonad indices were compared using a one-way ANOVA in R.

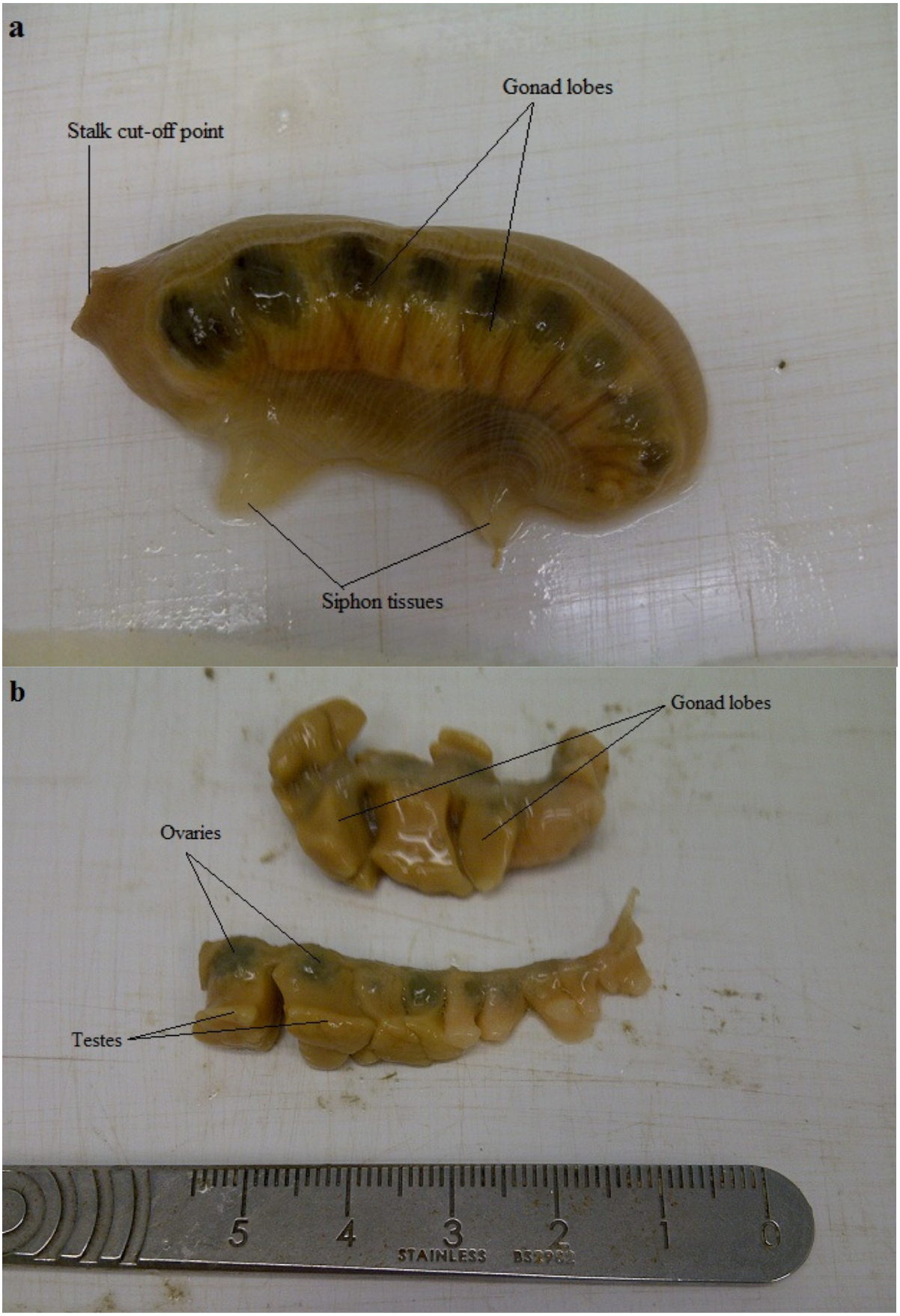


Figure 4.3. Photographs of *Pyura pachydermatina* a) Visceral mass, b) Rows of gonads, extracted during histological processing. These display an adult with mature ovaries and testes.

4.3 Results

4.3.1 Spawning experiment

During all seasons, spawning was observed in both the ‘single’ and ‘multiple’ treatments with the exception for ‘single’ treatment in fall where no spawning occurred (Figure 4.4). The individuals in the ‘multiple’ treatment spawned more often in all seasons with more than 80% of all replicates in each season producing offspring. The highest proportion of spawning observed in the ‘multiple’ treatment was during summer where all the replicates showed signs of spawning. The ‘single’ treatment in comparison was relatively lower in their proportions of replicates that showed signs of spawning. The proportion of spawning occurrence in the ‘single’ treatment was highest in winter with just over 55% and lowest in fall where no spawning was observed in any of the replicates (Figure 4.4).

As previously mentioned in Chapter 3, larvae were usually observed either on the third or seventh day post experimental setup. Although all eggs and larvae were counted, there was no way of telling if the larvae observed were the true proportion of viable offspring. This was because during counting, the eggs were usually at various stages of maturation and the exact times of spawning were not recorded.

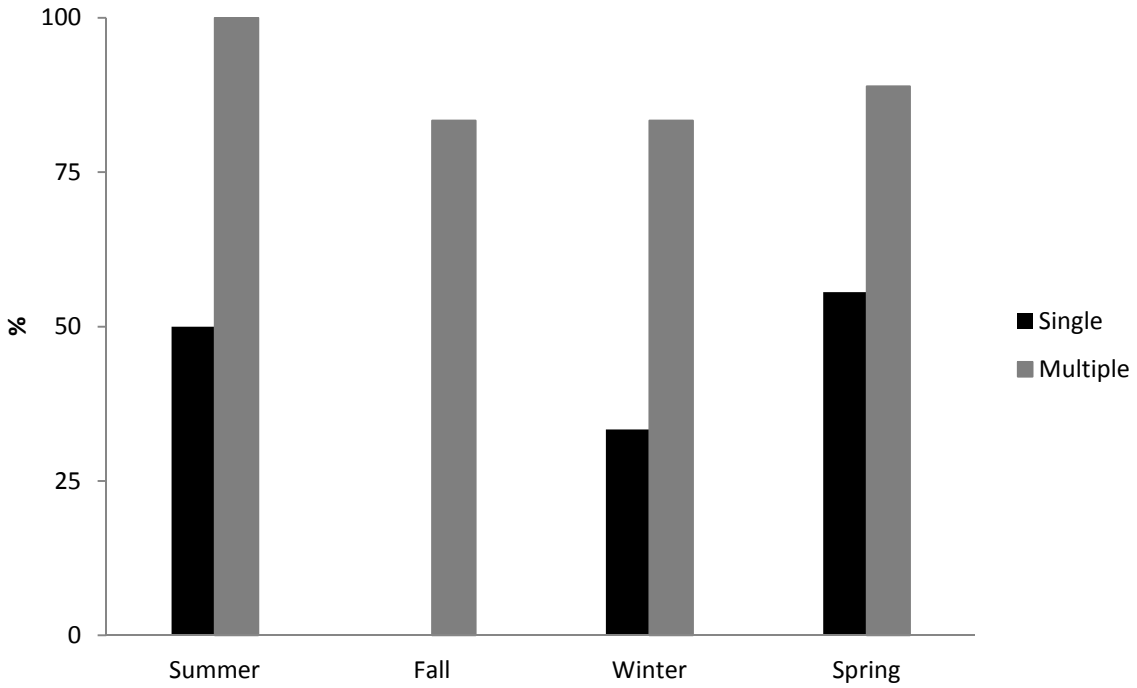


Figure 4.4. Proportions of containers/replicates within which, single or multiple individuals of *Pyura pachydermatina* were observed spawning during the different seasons.

4.3.2 Recruitment

Due to the rough conditions of the sea, some of the settlement plate rigs were either lost or damaged. Even though these were later replaced and the experiment resumed, there were a lot of gaps in the data. *Pyura* settlers were not observed in any of the plates retrieved during the experimental period. Interestingly, some *Pyura* were found attached on either the PVC pipe or on the ropes. The settlement plates constantly had attachment and growth of bryozoans. In addition, high numbers of amphipods were also present on the settlement plates.

4.3.3 Gonad indices

Comparison between the two gonad indices (Equations 4.1 and 4.2) showed that in relation to gonad weight, visceral mass ($R^2 = 0.872$) is a better measure of body size than total body

weight (R^2 = 0.757) (Figure 4.5). The rate of gonad development in *Pyura pachydermatina* (Table 4.2) was given by the relationship between gonad weight (both rows of gonads) and weight of the visceral mass (Figure 4.5a). The rate of gonad development was proportional to the body size for the range of sizes tested in this study. This was shown by the linear relationship between gonad weight and weight of visceral mass with little variance especially in smaller individuals (Figure 4.5a).

Table 4.2. Regression of gonad weight on weight of visceral mass.

n	39
Range of values:	
(y) gonad weight	0.17 – 9.49 g
(x) weight of visceral mass	1.19 – 30.61 g
(± s.d.)	3.999 g (± 2.509)
(± s.d.)	12.298 g (± 7.282)
R^2	0.872
Regression equation	y = 0.321x + 0.041

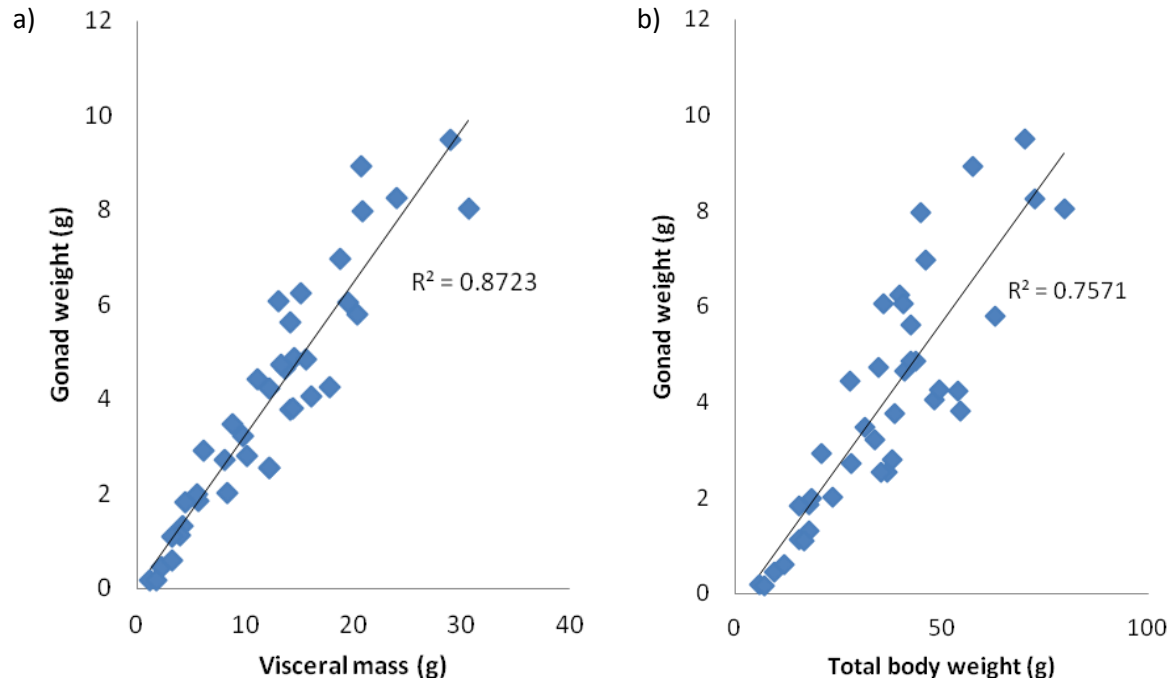


Figure 4.5. Relationships between gonad weight and a) visceral mass and b) total body weight for *Pyura pachydermatina* collected from May 2011 to February 2012.

The mean monthly gonad indices did not show any significant difference from one month to another ($F_{7,32} = 1.6648$, $p>0.05$) (Figure 4.6). There was a decline in the gonad indices from July to September 2011 which would indicate winter spawning in *Pyura pachydermatina*; however, the decline was not statistically different.

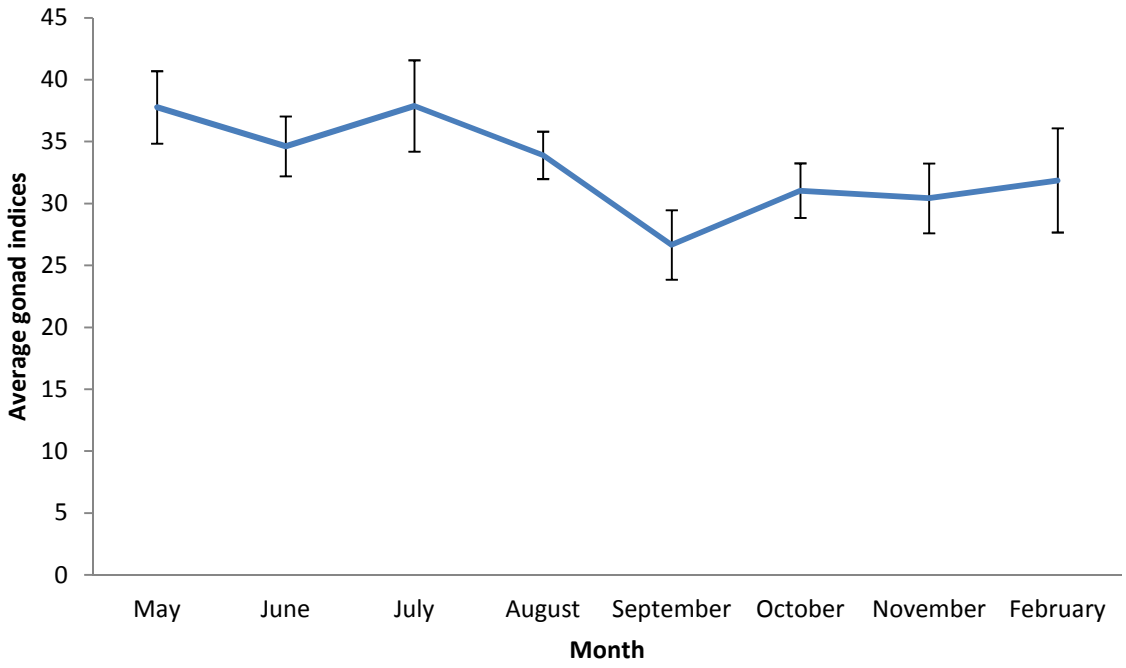
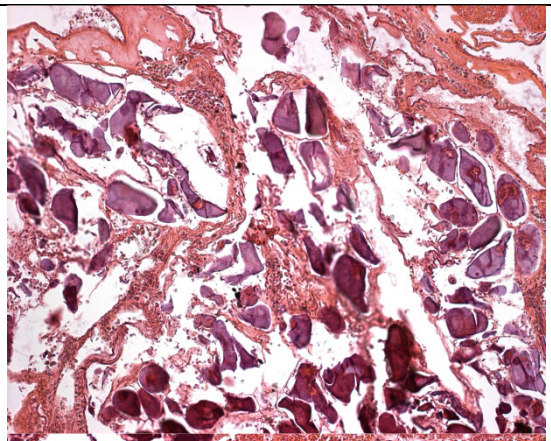
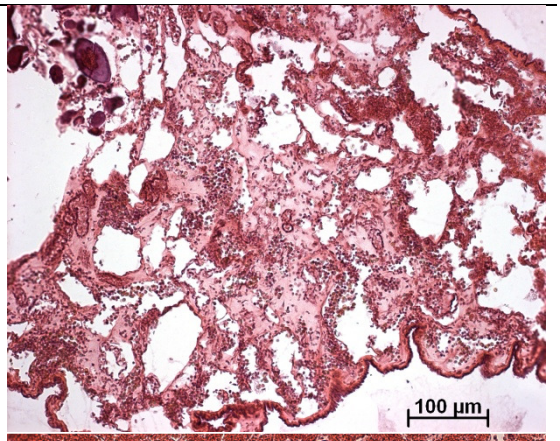
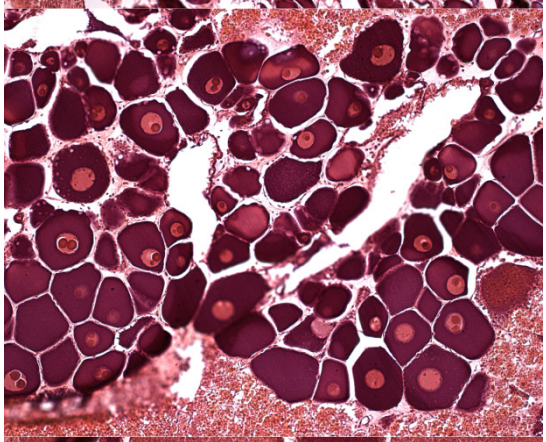
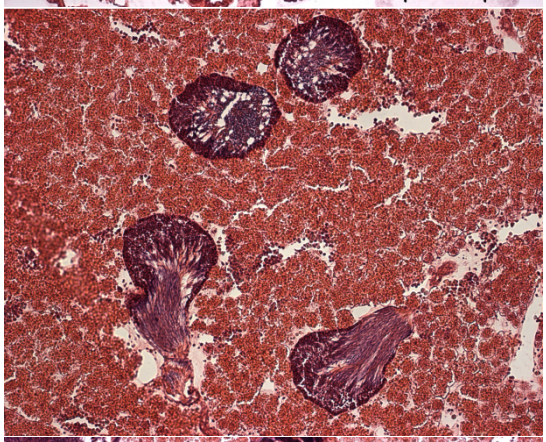
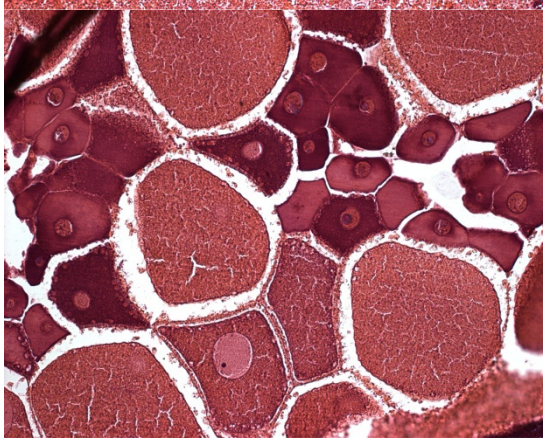
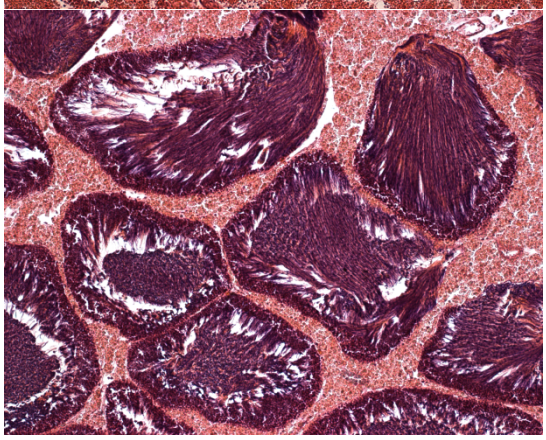


Figure 4.6. Monthly average of gonad index 1 (Equation 4.1) for *Pyura pachydermatina* (±S.E.) collected for histological processing from May 2011 to February 2012.

Stage	Ovaries	Testes
1		
2		
3		

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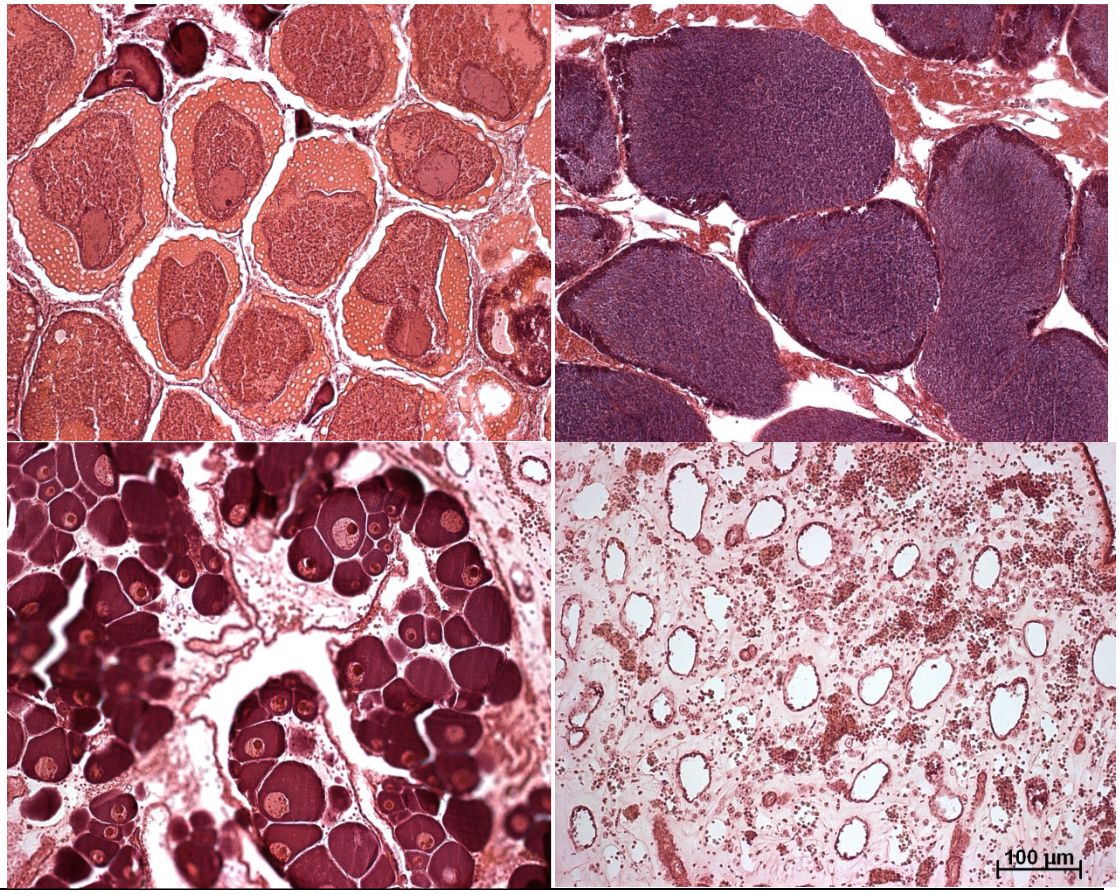

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Figure 4 7. Histological sections of all the developmental stages of *Pyura pachydermatina* ovaries and testes. These sections were viewed under a Zeiss Axio Imager M1 microscope and photographs were taken at the same magnification using a Zeiss AxioCam HRc camera. Full description of each stage is listed in Table A5.

The gonad development through the seasons was similar between the female gonads (ovaries) and the male gonads (testes) (Figure 4.7). During summer, the major proportion of both ovaries and testes was in stage 3 (late development). In winter and spring, the ovaries and testes were divided mainly into stage 3 and stage 4 (mature), although there was a small proportion of ovaries in stage 2 (early development) as well. The only exception seemed to be during autumn when there were no ovaries in stage 3 and there were some in stage 1 (inactive). This was slightly different to the testes development during autumn when there was a proportion in stage 3 and there was none in stage 1. Stage 5 (spawned/ redeveloping) was only observed in both female and male gonad tissues during fall (Figure 4.8).

Fisher's Exact test on gonad developments over seasons showed that ovarian development was dependent on seasons ($p = 0.002$), while testes development was not dependent on seasons ($p = 0.124$). χ^2 test showed that the presence of mature (stage 4) ovaries and testes together was independent of season ($\chi^2 = 0.971$, d.f. = 3, $p > 0.05$).

The minimum size (tunic length) of *Pyura* collected with gonad tissue in early development was 41 mm. All the specimens with less than 40 mm tunic length had not developed their gonad tissues enough to be included in this gonad index study. The last stage of gonad development observed in this study was stage 5 (spawned/redeveloping). There were no specimens which showed gonad tissues in regression phase.

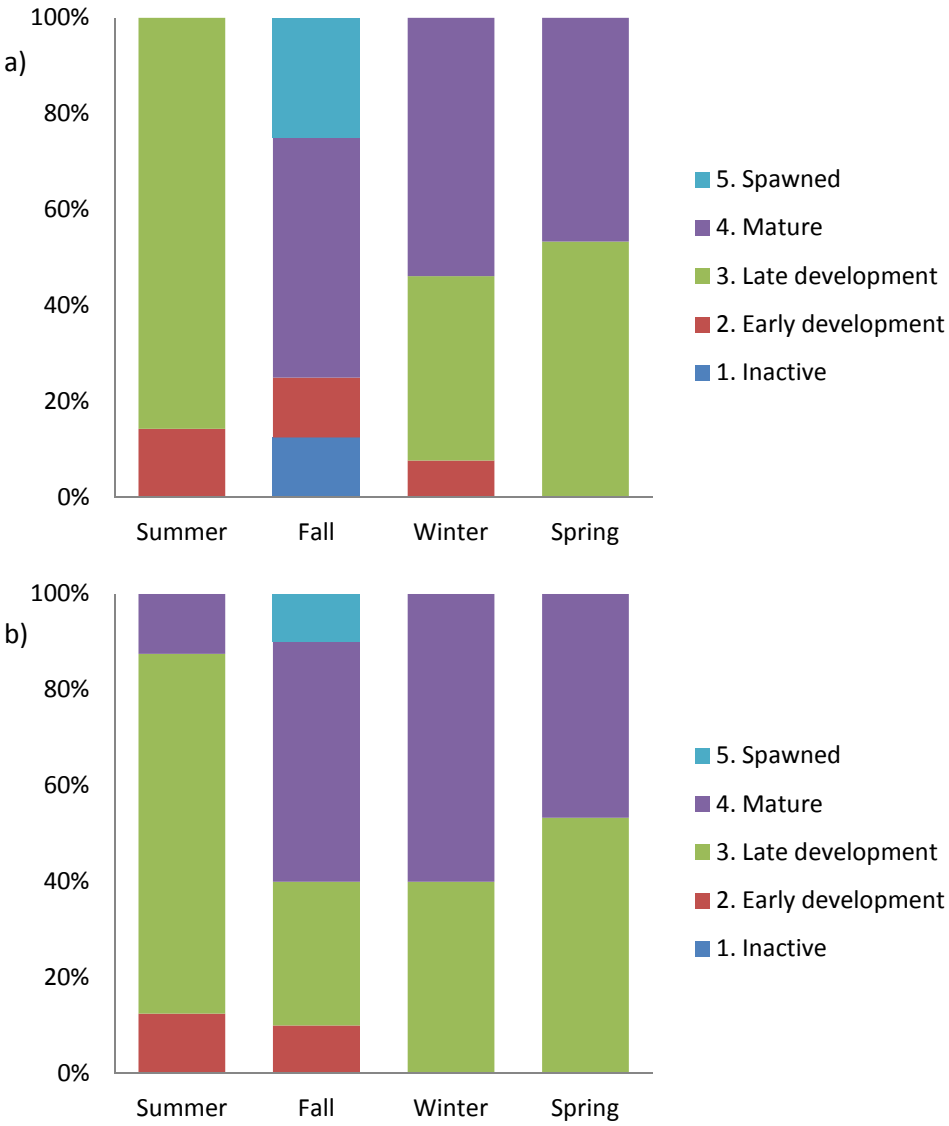

Figure 4.8. Proportions of a) female gonad tissues (ovaries) and b) male gonad tissues (testes) during all seasons. *Pyura pachydermatina* individuals were collected mostly from Camp Bay; and from Magazine Bay when conditions in Camp Bay were permitting.

4.4 Discussion

Spawning experiments carried out between January 2011 and February 2012 produced offspring in at least one replicate from each of the two treatments. This showed that *Pyura pachydermatina* has the potential to reproduce at any time during the year, although not all mature individuals would do so at the same time. This apparent lack of spawning synchronicity could further support the idea that *Pyura* was capable of self-fertilisation. Unlike those species which require synchronous spawning for cross-fertilisation (Manriquez & Castilla 2010), only one individual would be required to spawn for self-fertilisation to occur. Even though eggs were observed to be developing when counted and the presence of larvae indicated offspring viability, no aspect of the experiment tested for larval fitness and its ability to settle and undergo metamorphosis. Further study on this experiment could be done in the future by having more replicates and assessing larval fitness over the different seasons.

The recruitment plates did not recover any *Pyura* settlers over the course of one year. However, when the whole rigs were removed from the water at the end of the experiment, there were numerous *Pyura* individuals on the PVC pipe to which the recruitment plates were attached and also on the ropes holding the rigs together. The size of *Pyura* on the pipes and ropes were varied, suggesting that settlement on these structures must have taken place at different times during the year. The recruitment plates had a lot of bryozoans and hydroids growing on them. High numbers of amphipods were also regularly found on the plates from all sites. The dominating amphipods on these plates were *Caprella mutica* and *Jassa marmorata*. Several factors could have contributed to these observations including the exposure time and plate orientation. A similar study with similar result was carried out to

assess the settlement patterns of the ascidian *Microcosmus squamiger* in the Mediterranean Sea. However, instead of using PVC plates, plastic Petri dishes were used as the recruitment substrate and they were fastened to the rocky substratum instead of 'hanging' in the water column (Rius et al. 2009). Despite finding new colonisations of *M. squamiger* in the area, Rius et al. (2009) did not find any *M. squamiger* settlers in their recruitment dishes which were often colonised by algae.

The gonad index was developed as a measure of the relative proportion of the body of an organism allocated to reproductive tissue. Changes in the gonad index over time provided an indicator of the reproductive periodicity of a population (McClary et al. 2008) and have been widely used in the study of reproduction of marine invertebrates (Sahade et al. 2004). In this study, even though statistical analysis did not find any significant differences among the monthly mean gonad index, there were slight variations notably from the month of July to September 2011 (Figure 4.6). I suspect that the low number of samples used in this case may have contributed to the non-significance in the result and propose that a similar study should be carried out in the future with larger sample sizes to decrease the statistical errors. Studies using the gonad index must be carried out carefully because it is based on the assumptions that 1) there is an isometric relationship between gonad size and some measure of total size and 2) that gonads begin to develop at size zero (Ebert et al. 2011). A study on the sea urchin *Strongylocentrotus purpuratus* by Ebert et al. (2011) showed that gonad index is better modelled with a function that takes into account the size of initial gonad production.

Histological sections of *Pyura* gonads over a one year period showed that at any one season there were a combination of developmental stages of both the ovaries and testes (Figure 4.8). Generally stage 3 and stage 4 gonads dominated the population. These late development and

mature stages which were persistent throughout the year further supported the idea of year-round reproduction pattern for *Pyura*. However, there were some interesting observations made. For example, the proportion of mature testes was low and mature ovaries were non-existent during the summer months. This was interesting because the spawning experiment showed the highest incident of spawning during the summer months when the two treatments were combined (Figure 4.4). Low number of specimens collected during these months may have over-represented the gonads in stage 3 of development, while missing out the stage 4 altogether. Another interesting anomalous observation was the absence of stage 3 ovaries during autumn when there were those in stage 2 and stage 4. Again, low number of specimens may have contributed to these observations. A study in Ireland using histological sections of gonads in *Styela clava* showed a similar pattern to *Pyura* in that there were multiple stages of gonad maturation present in some months. However, *S. clava* in Ireland was found to have distinct spawning periods in late autumn and early summer (Parker et al. 1999).

Fisher's Exact test showed that ovary development was dependent on seasons, while the development of testes was not. χ^2-test on stage 4 (mature) ovaries and testes did not show any significant affect from seasons. This further supported the potential of *Pyura pachydermatina* to reproduce all year-round as mature gametes could be present at any one time regardless of seasons. Again, using *S. clava* as a contrasting comparison, stage 4 (mature) ovaries and testes are only present in this species in late autumn before spawning occurs and no mature gonad tissues are present by late summer (Parker et al. 1999).

Results and observations on the spawning capability and gonad stages throughout the year point to a conclusion that *Pyura pachydermatina* can reproduce year-round. Although no

spawning observations have been made in the field and there was a lack of settlers on the recruitment plates; the constant presence of mature gonads in adult individuals showed that this species is ready to spawn when required or when the conditions are ideal. This year-round reproduction makes *Pyura pachydermatina* unique to many other solitary ascidians, such as *Styela clava* or *Ciona intestinalis* which reproduce seasonally. Incidentally, *S. clava* and *C. intestinalis* are two NIS that are found in the Port of Lyttelton in the Banks Peninsula. Reproduction of *Pyura pachydermatina* during seasons when neither of the NIS is reproducing would present the offspring less competition, thus increasing the potential for successful establishment.

Chapter 5

Invasive Pressure

5.1 Introduction

5.1.1 Factors affecting invasiveness and invasibility

Sessile organisms of the intertidal rocky shores have to compete for space. This, together with predation, physical disturbances, resistance to physiological stresses, and propagule supply make up the structure of the ecological community (Roughgarden et al. 1988). *Pyura* being one of the common organisms in the intertidal rocky shores of the South-eastern coast of New Zealand have to deal with all the above factors to ensure the species' establishment in the community. Against the increasing pressure of urbanisation and NIS invasion; any one or more of these factors can change to either increase or decrease the fitness of this native species, ultimately leading to the success or failure of an invasion.

Physical habitat and disturbances play a large role in the competition between native and invasive species. The physical attributes of a habitat can have an effect on native species

resistance against NIS invasion. For example, distribution of the NIS clam *Nuttallia obscurata* in the north-eastern Pacific was restricted by the type of sandy substrates present at those local sites. The clams cannot find suitable refuge from predators in the densely packed substrate at lower tidal heights, where incidentally most of the native clams are found, and are therefore limited to only the higher tidal heights made up of loosely packed sand (Byers 2002). Physical disturbances such as wave action or hydrological current patterns can also restrict or promote NIS invasion. Castilla et al. (2004) suggested that even though the invasive ascidian *Pyura praeputialis* can grow and survive outside of the bay, their restricted distribution in the Bay of Antofagasta in Chile was due to a specific oceanographic retention mechanism. Another example is the microcosm experiments with protozoa and rotifers subjected to physical disturbances that resulted in decreased native species diversity and increased abundance of the invasive species (Kneitel & Perrault 2006).

Competition for space is another vital factor in the structuring of intertidal rocky shore communities as space is often the most limiting resource in that environment (Jackson 1977). Native species dominance in abundance and in competitiveness for gaining foothold of available space is a key characteristic in determining invasibility (Smith et al. 2004). This is because highly competitive and space-filling species can either enhance or reduce susceptibility to invasion depending on whether they create conditions conducive or stressful for the NIS. Clumping can be an example of the strategy employed by *Pyura* to gain advantage in the competition for space from other organisms. It allows them to use up a relatively small patch of area for a large number of individuals by having the same attachment point on the end of their stalks.

The effect of propagule supply on the invasiveness of NIS or invasibility of the recipient community is tricky to assess because of multiple stages in the life cycle of many marine organisms. To complicate things even more, some of these life stages are dependent on environmental factors (Connolly & Roughgarden 1999). *Pyura* and *S. clava* produce eggs which sink and larvae which are mobile. However, their immediate dispersal and transport are heavily reliant on physical oceanographic processes, predominantly water movement. The relationship between these oceanographic processes and the strength of species-benthic habitat interactions plays a large role in determining the success of establishment in the recipient environment. The abundance and frequency of these offspring transported into the environment, coupled with their ability to settle and grow are some of the key features in making up the recipient community.

5.1.2 Predation

One factor that has been extensively studied and has a big impact on ecosystem structure is predation (Connell 1961, Connolly & Roughgarden 1999, Grosholz et al. 2000, Harding 2003, Osman & Whitlatch 2004, Whitlatch & Osman 2009, Yamanishi et al. 2012). Predation by definition is the act of feeding behaviour by which an organism, the predator, kills and consumes another organism, the prey. Depending on the level of interaction, predators usually decrease and limit the abundance of prey species.

In relation to marine invasion, predation by a native species on an NIS species can slow down or stop invasion altogether, ultimately resulting in increased resistance against invasion for the native ecosystem. The native blue crab *Callinectes sapidus* of eastern North America predates on the introduced European green crab species *Carcinus maenas*. This predator-prey

relationship provides biotic resistance to invasion and prevents a southward spread and establishment of *C. maenas* in eastern North America (deRivera et al. 2005). In a different study on the same area with the same native blue crab, it was found that *C. sapidus* also predates on young Asian rapa whelk *Rapana venosa*. This resulted in a natural control of the introduced whelk population on the North American Atlantic coast (Harding 2003).

The opposite is also possible, whereby, the predator species is introduced and the native species is the prey. This can have devastating impact on the native prey species if there are no other factors that act negatively on the introduced predators. For example, the green crab *Carcinus maenas* significantly reduced the abundance of some native species in the coast of California. The native clams *Nutricola tantilla* and *Nutricola confusa*, as well as the native shore crab *Hemigrapsus oregonensis* all showed 5-fold to 10-fold decline in numbers only three years after the arrival of *C. maenas* (Grosholz et al. 2000). Feeding preference by native predator on native prey over invasive prey can enhance the invasiveness of the invasive prey species. For example, the sea urchin *Strongylocentrotus droebachiensis* prefers to feed on the native ascidian *Aplidium glabrum* over the invasive ascidian *Botrylloides violaceus*. This finding helped to explain the pattern of ascidian distribution and the success of *B. violaceus* as an invader in New England, USA (Simoncini & Miller 2007).

Predation can happen at any life stage of the prey's life cycle. Planktonic stage of marine invertebrates offspring are subjected to high mortality due to predation. Experiments using the ascidian *Styela gibsii* and the mussel *Mytilus edulis* showed differential predation on the larvae of polychaetes, echinoids, asteroids, gastropods, and cirripeds (Cowden et al. 1984). A study conducted by Osman and Whitlatch (2004) showed that predator-prey interactions involved predation of newly settled solitary and colonial ascidian juveniles by a variety of

predator species. They also showed that as a result of this predation on newly settled recruits, there were differences in the development and eventual species composition of the communities. A combination of differential predation and predation on early life stages has the potential to increase an ecosystem's resistance against invasion. For example, the non-indigenous ascidian *Ciona intestinalis* could not establish and could not become invasive in the natural benthic habitats of northern Chile because of the predation effects on their juvenile and adult life stages by native invertebrates and fish predators (Dumont et al. 2011). This was contrasted with the high abundance of a native ascidian *Pyura chilensis* that showed very little or no effect of predation.

5.1.3 Potential predators of *Pyura pachydermatina* early life stages

The recruitment plate experiment described in Chapter 4 did not yield any *Pyura* settlers on any of the plates retrieved. Instead, it was observed that there were constantly high numbers of amphipods on the settlement plate rigs. It led to the question being raised on whether or not those amphipods might be feeding on the early life stages of *Pyura*. By far the most common species of amphipods found were *Jassa marmorata* (Holmes 1905) and *Caprella mutica* (Schurin 1935). *J. marmorata* is a tubicolous amphipod species that originates from Europe but now has been recorded as far away as Mexico and New Zealand (Clancy 1997) (Figure 5.1). *C. mutica* or the Japanese skeleton shrimp has, as it name implies, originated from the northeast Asia and is currently distributed worldwide (Ashton et al. 2007, Cook et al. 2007) (Figure 5.2). Both these species are non-indigenous to New Zealand waters. The arrival period of *J. marmorata* is not clear, but *C. mutica* was first recorded in the Port of Timaru in 2002 (Woods 2012). These two species are commonly found in the same habitat

and it was established that the mud-tube colonies of *J. marmorata* provide a suitable attachment point for *C. mutica* (Schückel et al. 2010).


Figure 5.1. *Jassa marmorata* (www.boldsystems.org)

J. marmorata like all gammarids feeds primarily on detritus, but has been known to feed on smaller invertebrates. Caprellids are opportunistic omnivores that can filter feed with their antennae, graze on algae and detritus, scavenge and prey on smaller invertebrates, and even prey on their own species when food is scarce (Caine 1977, Guerra-García & Tierno de Figueroa 2009, Alarcón-Ortega et al. 2012, Woods 2012). Based on this information, it was possible for *C. mutica* and *J. marmorata* to feed on the early life stages of *Pyura* and contributed to the lack of *Pyura* recruits on the settlement plates.

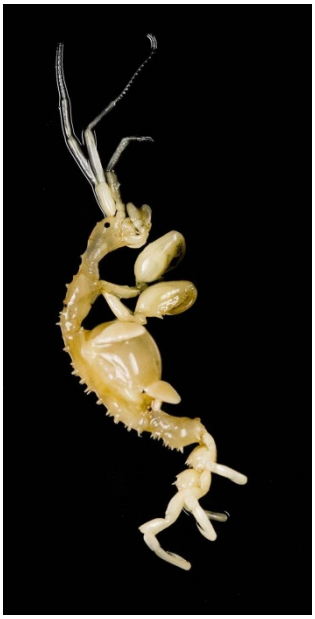
Figure 5.2. *Caprella mutica* (www.marinespecies.org)

5.1.4 *Styela clava* as a model for potential invasive species in Banks Peninsula

Recent introduction of *Styela clava* into the Banks Peninsula is of great concern, keeping in mind the invasive potential of this species to damage native ecosystem and also the fisheries industry (Bourque et al. 2007, Gust et al. 2008, Arsenault et al. 2009). Recent studies by Nutsford (2010) and also by Webber (2010) looked at the invasiveness of *S. clava* in the Banks Peninsula with regards to its reproduction, demography, and population projections. In this study, *S. clava* was used as the model organism of an invasive species.

5.1.5 Chapter objectives

Invasive pressure, specifically predation has the potential to shape local communities. The dynamic predator-prey interactions with regards to invasive and native species are crucial in determining the success or failure of an invasion. This chapter attempted to answer the following questions:

- Does *J. marmorata* or *C. mutica* feed on Pyura offspring? If so, at what early life stage?
- Is there any difference in predation of different life stages between the two predators?
- Is there any feeding preference from either predators on *P. pachydermatina* and *S. clava* offspring?

Using the information from studies by Nutsford (2010) and Webber (2010), I also attempted to predict possible future interactions between *Pyura* and *S. clava* in Banks Peninsula.

5.2 Methods

An experiment was carried out from December 2011 to February 2012 to assess the effects of predation on *Pyura* early life stages by amphipods. The two amphipod species used were *Jassa marmorata* (Holmes 1905) and *Caprella mutica* (Schurin 1935). These potential predators were chosen based on their high densities on the settlement plate rigs described in Chapter 4. Some preliminary experiments were carried out prior to starting the predation experiment. These were done mainly to find out if the chosen predators actually feed on *Pyura* offspring or not; and to find the most suitable surface for *Pyura* settlers to be used for the experiment.

5.2.1 Preliminary study

Nine plastic Petri dishes were set up with ten *Pyura* eggs in each dish filled with filtered sea water. Two *J. marmorata* were added to three of those dishes and two *C. mutica* were added to another three. The remaining three dishes were left without predators as a control. The numbers of remaining eggs in each dish were then counted after eight hours. Reduced numbers of eggs in Petri dishes containing predators were taken as a sign of predation.

Several different surfaces of the Petri dish were tested for the best one to be used for experiments using *Pyura* settlers. Three different factors were tested which were smoothness or roughness of the surface, light or dark surface, and upward or downward facing surface. Sandpaper was used to roughen up the surface of the Petri dish and black masking tape was used to darken the surface. Downward-facing Petri dish were set up in a beaker and suspended upside down with strings approximately about 1-2 cm from the bottom of the beaker. All Petri dishes used for this experiment were immersed in sea water for one day

before use. More than 200 *Pyura* larvae were added to each Petri dish and left to settle for three days. There were three replicates for each treatment. After three days, the unattached or dead *Pyura* offspring were removed by gently shaking the Petri dish and tipping the water out. The sea water was replaced and the number of settlers was counted. The first factor tested was smoothness or roughness of the surface. Visual observations made showed a clear difference with the roughened surface performing better for *Pyura* larvae to settle compared to the smooth surface. Therefore only roughened Petri dishes were used to test the other two factors for light or dark surfaces and upward or downward-facing surfaces.

5.2.2 Predation experiment

The early life stages of *Pyura* tested were the eggs, larvae, and settlers. The experiment was carried out in plastic Petri dishes with roughened surface filled with filtered sea water. The Petri dishes for experiments involving settlers of *Pyura* were preconditioned by immersion in sea water for at least 24 hours.

There were three treatments with two single species predators and a mixture of the two on the three *Pyura* life stages, giving a total of nine different treatments. Each treatment had eight replicates with an additional eight replicates of controls which contained no predators. For single predator treatments, three individuals of the predator were used in each replicate; whereas two individuals of each predator species were used in the replicates of the mixture treatment. 50 eggs and 50 larvae were used for each treatment replicate for these two life stages. As for treatments on *Pyura* settlers, more than 200 eggs were placed in each Petri dish and left to develop for three days. After three days, the sea water was tipped out leaving behind the *Pyura* settlers. The sea water was replaced and the number of settlers was counted

and recorded. This procedure yielded between ten to 40 *Pyura* settlers for each treatment replicate. Proportions were used in calculations involving the three life stages. Monitoring and counting of the *Pyura* early life stages were done at 5 hours and 24 hours after the predators were put into the Petri dishes. The number of *Pyura* offspring left in the Petri dish at the time of counting was taken as a measure of survival and the missing ones were attributed to the effects of predation. A GLM with quasibinomial error distribution was used to assess the proportion data among the different life stages of *Pyura* offspring and among the different predation treatments. ANOVA were done on mean proportions of survival in treatments for each individual life stages. When the treatment effects are significant, a Tukey's HSD test was performed to find out the significance among all treatment interactions. All statistical analyses were done in R (R Development Core Team 2011).

As an extension, another predation experiment was set up using the larvae of *Styela clava*. This was to compare between a native and invasive species and if the predator has a preference for one species over the other. We had difficulties in getting *S. clava* to spawn and as a result only had a limited number of offspring. It was then decided that only one predator species would be used in this case. The predator used for this was *J. marmorata*. This experiment was only done with larvae as eggs of *S. clava* were difficult to obtain before they developed into larvae. The settlers of *S. clava* did not survive for long after attachment and metamorphosis of larvae, so it was decided that this experiment would not be done on settlers. Eight replicates of the treatment were set up with three *J. marmorata*, 25 larvae of *Pyura* and 25 larvae of *S. clava* in each Petri dish. Another eight dishes with the same numbers of larvae were set up as controls with no predators. There were not enough *S. clava* collected for this experiment and treatments with only *S. clava* offspring were not carried out. Paired t-test was done to compare the effects of predation on *Pyura* and *S. clava* larvae.

5.3 Results

5.3.1 Preliminary study

After eight hours, the results from the nine Petri dishes were conclusive. Six of the dishes with predators had no eggs left in them, while the other three control dishes still had their original number of eggs. This showed that both *J. marmorata* and *C. mutica* do feed on *Pyura* eggs.

The highest number of settlers was found in the upward-facing roughened-surface Petri dish (Figure 5.3). The darkened and roughened Petri dish facing upward also produces a high number of settlers, similar to the non-darkened ones. The Petri dish with smooth surface on average had less than half the settlers then the rough surface ones. The two treatments where the dishes were facing downward did not give any settlers at all (Figure 5.3). Therefore it was decided that a non-darkened roughened-surface Petri dish would be used for the rest of the predation experiment on *Pyura* settlers. The result of no settlers in all the downward-facing Petri dish was interesting because of two reasons. Out in the rocky shores in all my study sites, *Pyura* could be found commonly attached sideways or upside-down under rocky overhangs with no apparent preference for attachment orientation (pers. obs.). It was also observed that the larvae of *Pyura* were very mobile and capable of swimming upwards towards the water surface in the beaker during the experiment. Their apparent preference for settling at the bottom of the Petri dish should be studied further.

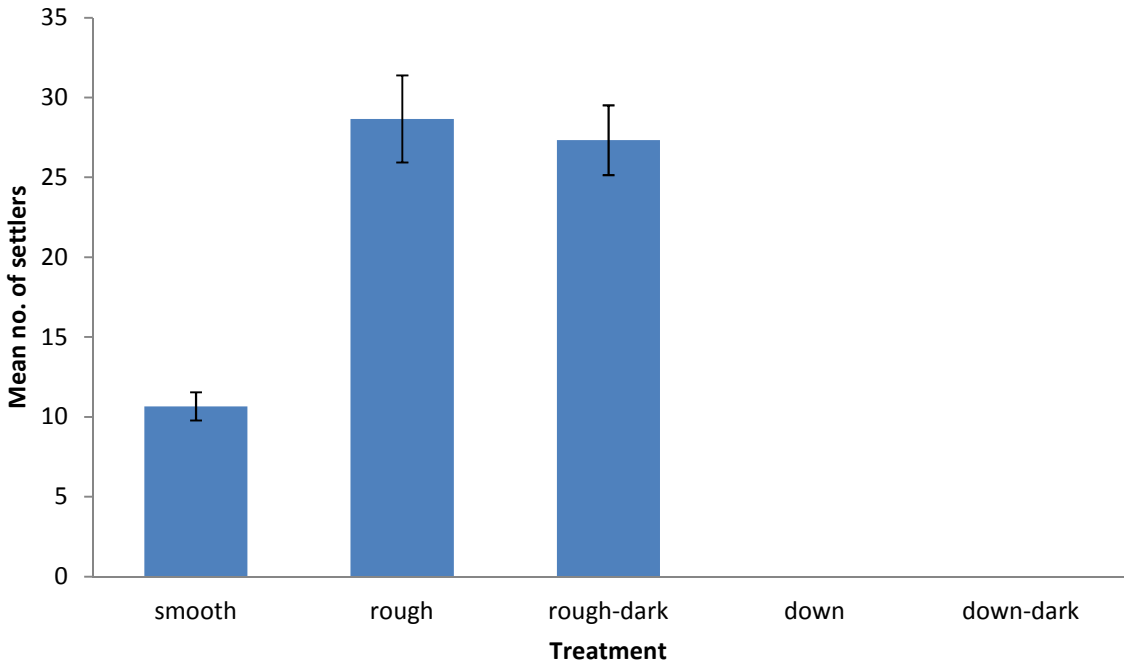

Figure 5.3. Mean number of *Pyura pachydermatina* settlers (± S.E.) after 72 hours on various Petri dish surfaces.

5.3.2 Predation experiment

It was established from the preliminary study that the two species of amphipods chosen feed on *Pyura* eggs. The results of the full predation experiment showed that the predators feed on eggs and larvae of *Pyura*, but not on the settlers (Figures 5.4 and 5.5).Predation on eggs by *Jassa marmorata* were slower initially when compared to *Caprella mutica*. After five hours of the experiment, *J. marmorata* showed no signs of predation of *Pyura* eggs (vs. control $p>0.05$), whereas *C. mutica* showed signs of more aggressive feeding on the eggs with less than 60% of the eggs remaining (vs. control $p<0.05$) (Figure 5.4). A mixture of both predators also showed signs of predation after five hours (vs. control $p<0.05$), although not significantly different to the treatment with only *C. mutica* ($p>0.05$) (Figure5.4). The larvae were predated upon relatively quickly with only 55-65% remaining after five hours in all the

treatments with predators (vs. control $p<0.05$). There were no significant differences among the proportions of larvae survivors in three treatments with predators after five hours.

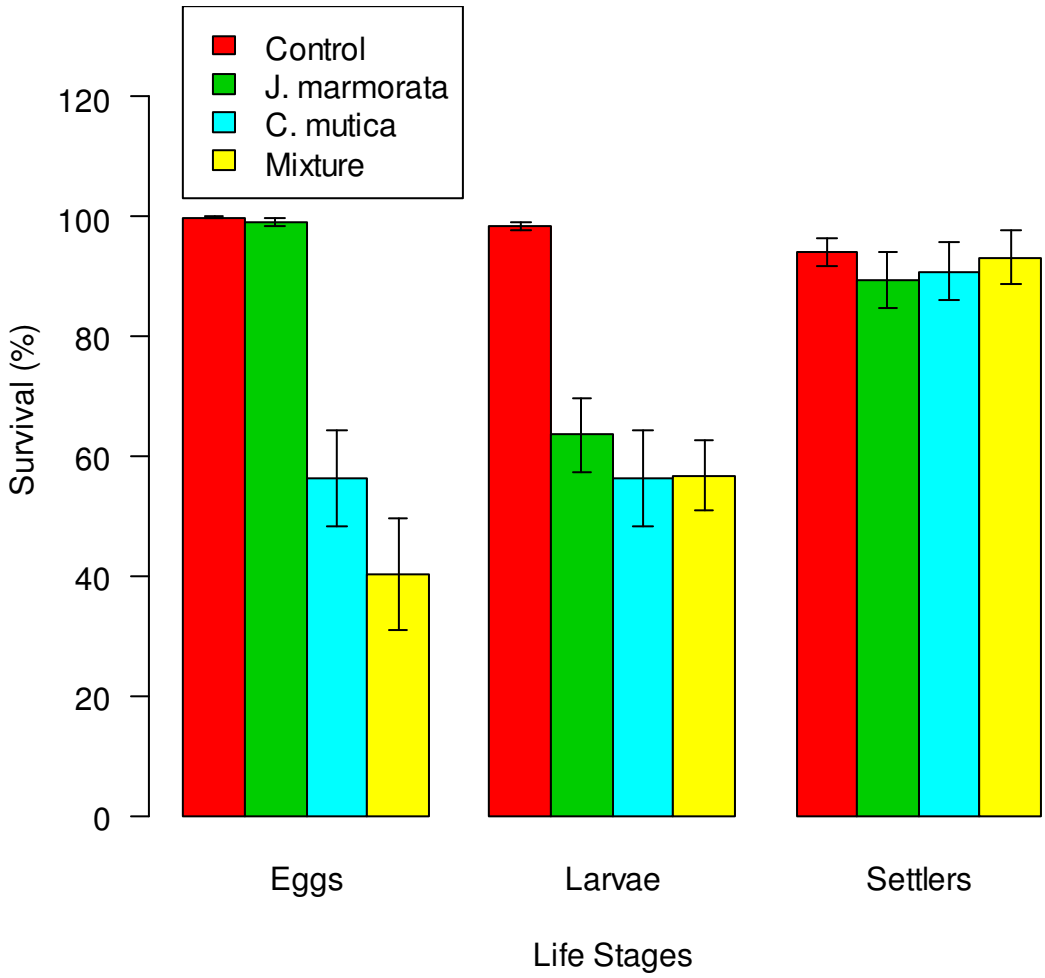

Figure 5.4. Proportion of *Pyura pachydermatina* offspring remaining after five hours (± S.E.).

After 24 hours of the experiment, there were further reductions in the number of remaining eggs and larvae in the predator treatments. The remaining number of eggs was just over 50% in the treatment with *J. marmorata*, just over 5% with *C. mutica*, and less than 1% with a mixture of both predator species (Figure 5.5). It was clear that *C. mutica* was a more efficient predator of *Pyura* eggs in this case compared to *J. marmorata*. From the mean proportion of

remaining eggs after 24 hours, the mixture of two different species of predators seemed to increase predation; however this may not be entirely accurate (Figure 5.5). The treatment with a mixture of predators has one more extra individual predator compared to single predator species treatments (4 versus 3).

The predation of larvae by *J. marmorata* after 24 hours left on average just under 24% of the original number of larvae. In contrast, there was only slightly more than 5% of remaining larvae in the *C. mutica* treatment (Figure 5.5). Again, as with the experiments with the eggs, *C. mutica* was the more efficient predator on *Pyura* larvae compared to *J. marmorata*. The level of predation observed in the treatment with a mixture of predators on larvae was higher than that of *J. marmorata*, but lower than *C. mutica* (Figure 5.5). This result was different than that of the same treatment on *Pyura* eggs. The mixture of predators did not seem to increase predation on larvae even with the extra predator.

There were minimal reductions in the proportion of *Pyura* settlers after five and 24 hours in all treatments and they were not significantly different to one another, including the control (after five hours: $F_{3,44} = 0.3646$, $p>0.05$ and after 24 hours: $F_{3,44} = 0.4892$, $p>0.05$). It was concluded that neither of the predators feed on the settler stage and no further analysis was done on this life stage. All declines in the numbers of *Pyura* offspring in the control treatment were due to dead or disintegrated eggs, larvae, or settlers.

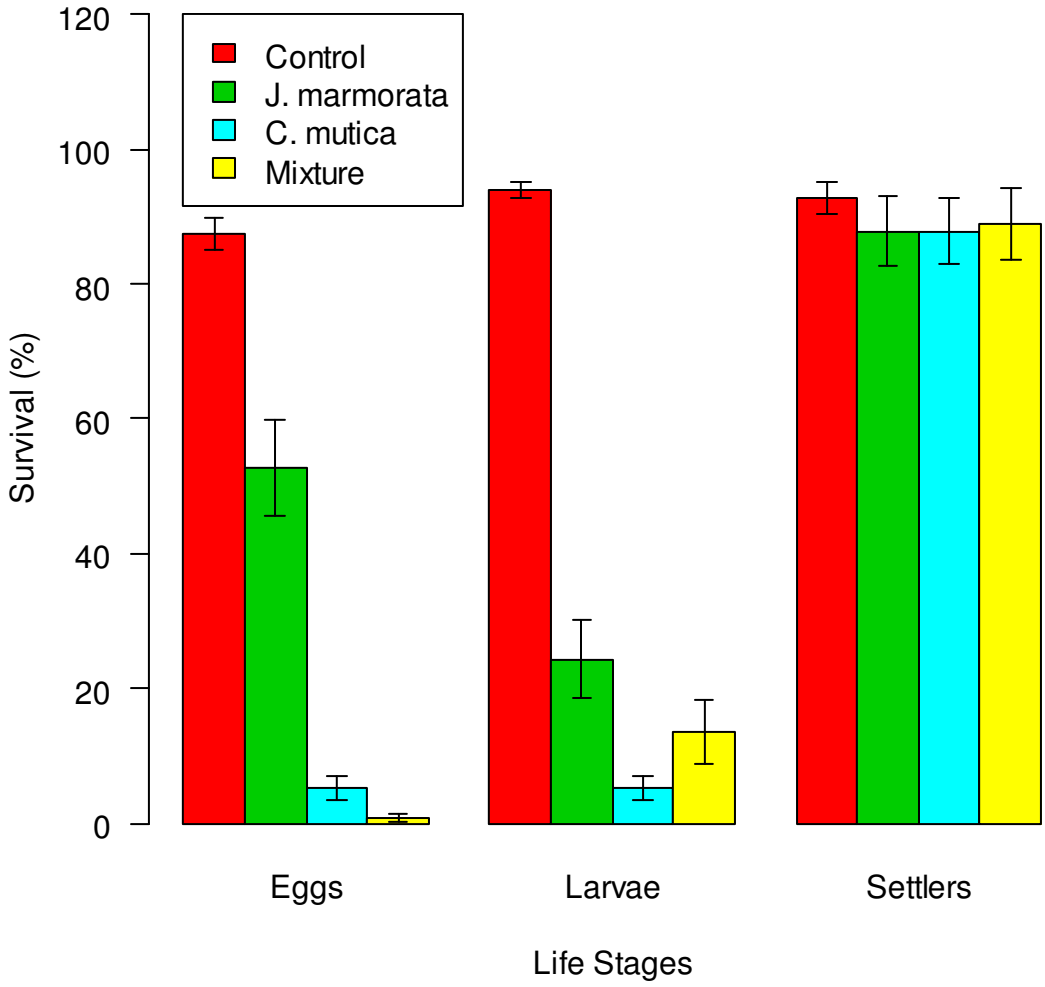

Figure 5.5. Proportion of *Pyura pachydermatina* offspring after 24 hours (± S.E.).

The experiment using a mixture of *Pyura* and *Styela clava* larvae with *J. marmorata* as the predator showed that after five hours, there was a significant difference in the survival proportions and there looked to be a feeding preference towards *Pyura* larvae (mean = 22.5, d.f. = 7) than *Styela* larvae (mean = 49.0, d.f. = 7) even though both were predated upon ($t = -5.9309$, $p<0.05$) (Figure 5.6). However, after 24 hours there was no significant difference between the numbers of remaining larvae of *Pyura* (mean = 17.5, d.f. = 7) and the larvae of *S.*

clava (mean = 20.5, d.f. = 7) (t = -1.8209, p>0.05) (Figure 5.7). Therefore the feeding preference that might be shown after five hours was not evident after 24 hours.

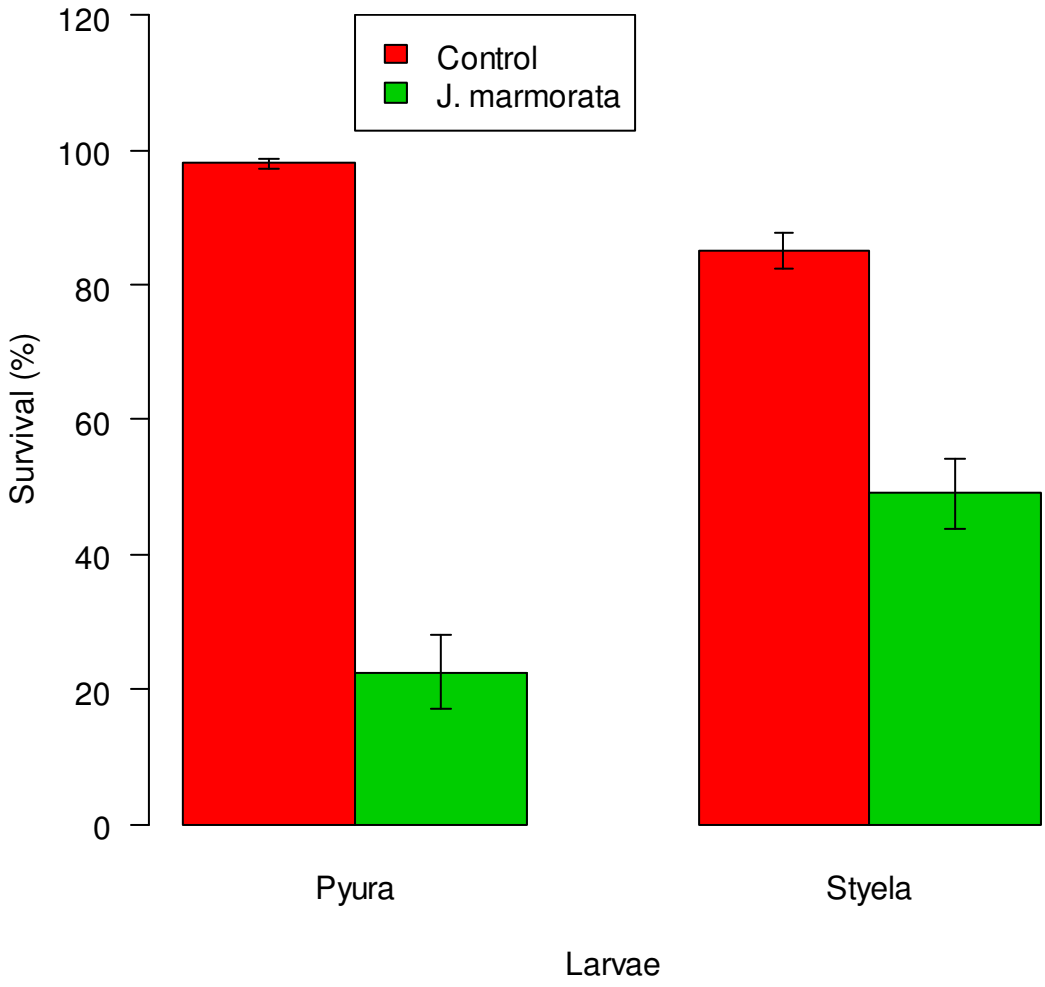

Figure 5.6. Proportion of remaining larvae of both *Pyura pachydermatina* and *Styela clava* after five hours (± S.E.).

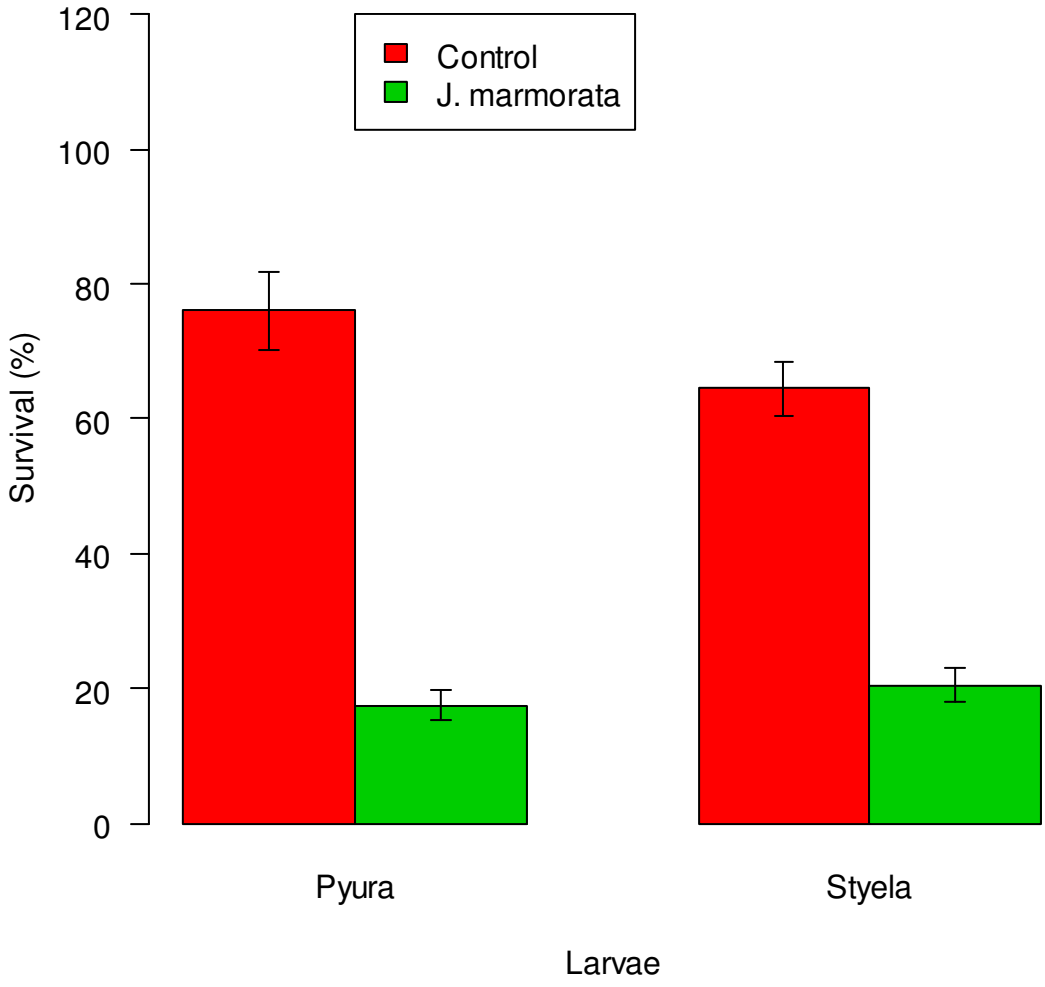

Figure 5.7. Proportion of remaining larvae of both *Pyura pachydermatina* and *Styela clava* after 24 hours (± S.E.).

5.4 Discussion

Preliminary experiment results on surface preference for settlement of larvae showed that *Pyura* larvae chose to settle on rough surfaces more readily compared to smooth surfaces. This further supported the idea that ascidian tadpole larvae are capable of discriminating finer surface details than just merely detecting hard substrates (Flores & Faulkes 2008). In their experiment using larvae of *Ascidia interrupta*, Flores and Faulkes (2008) found that the larvae preferred sandblasted surfaces over smooth surfaces and sandpaper-roughened surfaces. They then hypothesised that ascidian tadpole larvae have mechanoreceptive sensory neurons capable of sensing surface textures for ideal settlement.

Based on the results of the predation experiment, it was certainly possible that predation was the reason there was no *Pyura* recruits found on the settlement plates experiment. Significant declines in the numbers of both eggs and larvae in the treatments with predators showed that *J. marmorata* and *C. mutica* were efficient predators for these two early life stages of *Pyura* (Figure 5.5). I hypothesise that significant amount of predation on eggs and larvae of *Pyura* are happening in the local communities of Banks Peninsula. Currently there is very minimal data on the abundance and distribution of the two predator species used in this study (Woods 2012). Based on personal observation in my study sites, the distribution of these predators are patchy and they are showing no negative effects on the health of the local *Pyura* populations. However, the long-term effect of the predation is still not yet known.

As *J. marmorata* and *C. mutica* are not natives to New Zealand, further close monitoring of these two species are required to learn the long-term impacts on the local ecosystem. Non-indigenous predators have been known to cause degradative results not only on local communities, but also on the local fishery industries. Mussel farming is the dominant fishery

industry around Banks Peninsula. Although there have been no reports of negative effects on this industry by NIS invasion, it does not mean that it is immune to invasion in the future. Marine fishery of the venerid clams, *Katelysia scalarina*, in Tasmania was predicted to be significantly and negatively affected by invasion of the green crab predator, *C. maenas* (Walton et al. 2002).

Interesting results were obtained in experiments with *Pyura* settlers as they were not being predated upon. During larval attachment and metamorphosis into young recruits, they could have developed a resistance mechanism which protected them from predation. Numerous studies have been conducted to assess the defences of marine invertebrate offspring against predators (Lindquist et al. 1992, Lindquist & Hay 1995, 1996, Lopanik et al. 2006, Paul et al. 2007, Williams et al. 2011). Although most of the studies focussed on the larval stage of offspring development, the survey done by Lindquist and Hay (1996) on various marine invertebrate larvae resulted in different levels of palatability of those larvae to most generalised planktivores. Even though *Pyura* larvae in the predation experiment did not exhibit any signs of chemical defences to reduce their palatability against the two predators, the newly settled recruits certainly did. It is not yet known what chemicals are protecting these young recruits. In some ascidian species, high levels of acid in the tunic fluids and high levels of vanadium have been shown to deter predators (Stoecker 1980).

Although the full preference experiment on predation of *Pyura* and *S. clava* early life stages was not carried out; there was no preference shown towards the larvae of *Pyura* or *S. clava* by *J. marmorata* when both species were in the same treatment. This result meant that in presence of *Pyura* larvae, predation of *S. clava* larvae can be a form of natural control and increase the resistance of native community against *S. clava* invasion. However, depending

on the level of predation on *Pyura* early life stages, this can also reduce the abundance of this native species in the local communities. Further study should be carried out in the future that includes single species prey treatments (i.e. only *Pyura* and only *S. clava* offspring) and also other predator species. Predation experiments with larvae should also be conducted at the same time because larval activity decreases with time after hatching out from the eggs (pers. obs.). This is to remove any potential bias that may be associated with predation on more active larvae compared to less active ones.

Styela clava is an ideal species to be used as a model NIS in this study as it has the potential to invade more natural habitat around the Banks Peninsula. Although this species was identified as early as 2002 in the Port of Lyttelton (Gust et al. 2008) and there has been evidence of multiple incursions into the area (Goldstien et al. 2010), its distribution has been highly restricted to the port and its neighbouring bays (Goldstien & Flikac pers. comm.). The study on original *S. clava* population inside the port showed that it was either static or in decline, which was contrary to the original expectations (Webber 2010). The reproductive output of *S. clava* could not compensate for high mortality rate of its early life stages (Nutsford 2010). This was further supported by observations in the port and neighbouring Magazine Bay in the summer of 2011/2012 when there were very few *S. clava* individuals available for use in the predation experiment. However, Webber (2010) also mentioned that this small restricted population in the Port of Lyttelton could be acting as a stepping stone for the spread of this species to the Banks Peninsula. Mussel farms are also vulnerable because if the spread of this species reaches the farms, they would be provided with more conducive conditions for survival and growth (Nutsford 2010).

It is difficult to predict what will happen to the native *Pyura* populations should *S. clava* become invasive and prominent in the future. Predation on *S. clava* larvae by *J. marmorata* in this study showed that it may offer a mechanism for increased resistance against invasion, but at the same time predation effect on *Pyura* larvae should be investigated further. The clumping exhibited by *Pyura* may also increase its resistance against invasion by reducing the spatial requirement for population establishment. The projected decline of *S. clava* population in the Port of Lyttelton may mean that *Pyura* may never have to compete with invasion of *S. clava*, but with the increased shipping traffic in the area, it is possible that one day *S. clava* may become invasive throughout the rocky shores of the Banks Peninsula.

Chapter 6

General Discussion

6.1 Overview

This study investigated the general demography and the life history strategies of a native ascidian *Pyura pachydermatina* that is under increasing stress from urbanisation and invasion. The aim was to describe the current status of *Pyura* population around the Banks Peninsula and to explore the potential of *Pyura*'s reproduction strategy to increase its resistance against invasion. The close proximity of Christchurch, the largest city in the South Island, to the peninsula means that urbanisation is a constant and ever present threat to the native biodiversity. Environmental pollution is one example of the effect of urbanisation. For instance, chemical discharge into the sea can have an adverse effect on the life cycle of marine organisms as demonstrated by Gallo et al. (2011) who showed that the reproduction mechanisms of the ascidian *Ciona intestinalis* was negatively altered when exposed to four different heavy metals. In addition, the Port of Lyttelton is located in one of the many bays of the Banks Peninsula and, as the main trading port for the South Island; it receives a high volume of ship traffic on a daily basis. A biological survey done in 2006 in the Port of

Lyttelton resulted in 150 native species, 22 cryptogenic species, and 20 non-indigenous species (NIS) being identified (Inglis et al. 2006). The effects of these NIS on the native species have not yet been studied yet, but many studies on biological invasions show that invasive species have the capability to alter community assemblages and impact negatively on the native species (Blum et al. 2007, Bullard et al. 2007, Dijkstra et al. 2007b, Sax & Gaines 2008).

Native species should have an evolutionary advantage over NIS for establishment because they are better adapted to local conditions (Byers 2002). Several studies looked at the different strategies employed by native species to increase their resistance to invasion (Baltz & Moyle 1993, deRivera et al. 2005, Maron & Marler 2007, Dumont et al. 2011). One widely accepted idea is that diversity increases community resistance. Some studies in the past have argued that higher biodiversity in a community results in increased resistance against invaders (Stachowicz et al. 1999, Stachowicz et al. 2002). However, these studies often used multiple species and looked at the community dynamics as the mechanism of increased resistance and did not focus on a single species or particular strategies used for increasing resistance (Dunstan & Johnson 2007, Maron & Marler 2007). Other studies investigated habitat attributes (Byers 2002), community interactions (Grey 2011, Yorke & Metaxas 2011), and predation (Dumont et al. 2011, Yamanishi et al. 2012) as mechanisms for increased resistance to invasion. Again, these studies did not focus on the strategies employed by a native species, but instead looked at external factors that might increase the native species' resistance.

Pyura is a native solitary ascidian with a distribution in the south of Australia and south east of New Zealand (Egan 1984b, Schiel & Hickford 2001). To date, few studies have been

conducted on *Pyura*, and those that have been conducted focused on its early life development or its seasonal occurrence in relation to a suite of organisms which reside in/on *Pyura*, such as the nemertean *Gononemertes australiensis* and the copepod *Pachypygus australis* (Anderson et al. 1976, Egan 1984b, a). Lack of updated data on the local population structure of *Pyura* and contrasting information on its reproduction biology prompted the start of this study. The following discussions aim to describe the current local population demography of *Pyura*; and shed some light on strategies employed by *Pyura* to resist the potential negative impacts from urbanisation and invasion.

6.2 Demography of *Pyura pachydermatina* in Banks Peninsula

Three populations of *Pyura* around the Banks Peninsula were surveyed over a period of one year to investigate their abundance and size frequency. Results from the survey suggested that these populations are distinct and different from one another. However, it must be noted that the survey was carried out at the three different sites only from the lower tide line up to a depth of one metre subtidal. The *Pyura* individuals at greater depth were not included in this study. A previous abundance survey in 2001 done on two different sites on the northern side of Banks Peninsula (Schiel & Hickford 2001) produced lower abundances than the two sites on the northern side of Banks Peninsula in this study. The survey by Schiel and Hickford (2001) was carried out only once, whereas this study comprised a full-year data set for the survey. Several reasons could explain the difference in abundances of *Pyura* at these sites. It could be that they are independent populations with historically different abundances. It could also be the result of different surveying technique whereby I only measured to a depth of one metre and not to a depth of 15 metres like the 2001 survey. However, this is unlikely to be the case as there is no indication that this species is found more in shallow water

compared to deeper water (pers. obs.). Another possible reason is that there has been an increase in the general population of *Pyura* in northern Banks Peninsula over the last decade. If this is a true increase in the general population of *Pyura*, it would mean that the population is thriving and there has been no negative impact on this species from urbanisation or invasion in the areas studied. Even though none of the study sites are currently affected by urbanised structures, the sites surveyed by Schiel and Hickford (2001) are most likely covered in rocks as a result of the earthquakes that struck the area in the past two years. I suggest the use of genetic technology to study the connectivity among the local populations of *Pyura* at different study sites to determine the relationships among them.

Comparison of the average sizes of *Pyura* over each season in the three sites showed a general trend of Wainui having the largest individuals and Camp Bay having the smallest individuals (Figure 2.9). Growth and body size of an organism are usually linked to the amount of food availability (Winemiller & Rose 1993). This would suggest Wainui has the most nutrient-rich water followed by Pigeon Bay and Camp Bay. Another possibility is the effect of pollution on the species' growth. The close proximity of Camp Bay to the Port of Lyttelton may exposed the native species to higher levels of pollution compared to Pigeon Bay which is further away and Wainui which is on the other side of the peninsula. Future studies should investigate the water quality and amount of production for the different sites to relate them with the growth of native species.

6.3 Reproduction of *Pyura pachydermatina*

It is now conclusive that the hermaphroditic *Pyura pachydermatina* is capable of self-fertilisation. This mechanism of breeding was also readily used by this species all year round,

as evident from the results of the spawning experiments conducted in this study. At least one of the three replicates of the 'single' individual treatment spawned every time the experiment was carried out with viable larvae present one or two days post-spawning. Self-fertilisation is advantageous for a sessile organism with short-distance dispersal potential such as *Pyura* (Lambert 2005a, Castilla et al. 2007). Isolated individuals, as is commonly found in man-made structures such as wharf pilings or floating pontoons may be distributed far from the next individual and in some cases they are so isolated that the chance for cross-fertilisation is close to zero (Marshall 2002). Increased urbanisation opens up more of these man-made structures as potential habitats and the numbers of individuals isolated on these structures are likely to increase (Connell & Glasby 1999, Holloway & Connell 2002). Self-fertilisation allows these isolated individuals to keep breeding and gives the species a chance to develop the following generations to ensure persistence. This may be an example of a demographic Allee effect where the isolated population produces a positive association between per capita population growth and population size.

It was also established that *Pyura pachydermatina* is capable of reproduction all year round. This was evident from the spawning experiments and the study on gonad histology. *Pyura* was able to spawn every time the spawning experiment was carried out every six weeks over the one year of this study. Mature male and female gonads containing mature sex cells were also found every month when the histological study was done. This finding disputes the study by Egan (1984b) which stated that *Pyura* has a seasonal reproduction period with a peak of spawning events observed in late autumn and early winter. The implications of year round reproduction are vast and extremely interesting to a global audience. Many temperate marine invertebrates have a summer reproduction period when the water temperature is higher (e.g., *Pyura praeputialis* (Manriquez & Castilla 2010) and *Styela clava* (McClary et al. 2008,

Nutsford 2010). Reproduction at any other seasons (i.e., autumn, winter, and spring) would leave a species vulnerable to competitive exclusion from species that reproduce during the summer period, further exacerbated by the die-off of adults during the summer when other species are colonising these vacated spaces. However, a year-round-reproducing species might have an advantage for the establishment of their offspring when there is less competition for space or other resources from other offspring of summer-reproducing species. The solitary ascidian, *Herdmania momus*, was found to reproduce all year in the Red Sea but was seasonally restricted in the Mediterranean Sea (Shenkar & Loya 2008), suggesting that reproductive period is strongly influenced by environmental conditions and food availability.

This further calls for a future study which looks at the spatial pattern of reproductive output, water quality, and food availability around Banks Peninsula. If the conditions and food availability are more favourable in some sites more than others (e.g., at sites further away from the port or from urbanisation); then it is possible that only certain populations of *Pyura* exhibit year-round reproduction.

6.4 Invasive pressure on *Pyura pachydermatina*

Invasive pressure is a very real threat to native species of Banks Peninsula such as *Pyura*. A few years ago, 20 NIS and more than 20 cryptogenic species were identified from a survey done in the Port of Lyttelton (Inglis et al. 2006). Some of these NIS are notorious invaders in other parts of the world. For example, the clubbed tunicate *Styela clava* was a major invader species and became a pest which decimated the aquaculture and fisheries industry in Prince Edward Island, Canada (Bourque et al. 2007, Arsenault et al. 2009). The initial mechanism of transport of these NIS into a new environment is usually by shipping whereby the organism

either fouls the hull of the ship or gets carried over in the ballast water and discharged at the new location (Carlton 1999, Bax et al. 2003). The level of impact an invasive species has on the native biodiversity varies depending on the invasiveness of the species and the invasibility of the recipient environment. Studies by Nutsford (2010) and Webber (2010) on *Styela clava* in the Port of Lyttelton predicted that this species was in a static or declining phase, but they did not rule out the possibility of the species using the port as a stepping stone to invade and establish in the neighbouring bays. Some invasive species have also been documented to follow "boom and bust" cycles where the numbers can increase or decrease in very short spans of time and often unexpectedly (Freeland 1986, Boyd & Barbour 1993). Therefore, even though currently the population of *S. clava* in the Port of Lyttelton and neighbouring bays seem to be in decline (Goldstien & Flikac, pers. comm.), the population could as easily bounce back and become invasive very quickly, especially with the ever increasing amount of ship traffic in the area. When a species like *S. clava* becomes invasive, it outcompetes the native species for resources such as available space for settlement.

Other mechanisms that can contribute to invasive pressure on a native species is the pressure from predation. When an invasive species is also a predator to a native species, then the adverse effect towards the native species would be more prominent if there are no other factors that are controlling the predator. For example, the green crab *Carcinus maenas* in a Californian bay predates on native invertebrates and reduced their abundance significantly over a nine-year study period (Grosholz et al. 2000). The predation experiment in this study used two species of non-indigenous amphipods, *Jassa marmorata* and *Caprella mutica*, and their predation effects were tested on early life stages of *Pyura pachydermatina*. The results were that the two amphipods are efficient predators of the eggs and larval stages of *Pyura*, but not of the settlers. This observation may partially explain the lack of settlers found on the

settlement plates deployed in Camp Bay and Pigeon Bay. The experiment also showed that *Pyura* develop a defence mechanism during larval metamorphosis that deters the predators so that they are not preyed upon at that life stage. Ascidians are known to be rich in chemicals especially in their tunic (Stoecker 1980, Lindquist et al. 1992), thus the defence mechanism exhibited by the settlers in the predation experiment may be related to the tunic formation and the chemicals associated with it. Further study should be conducted to investigate this potential resistance mechanism against predators. The predation experiment was done on a small scale and even though it was established that the non-indigenous amphipods were efficient predators of *Pyura* eggs and larvae; the impact on the overall population of *Pyura* in the local communities was not tested.

6.5 Conclusion

Overall, this study has explored the life-history strategies employed by the native ascidian *Pyura pachydermatina* and assessed its potential resilience against the increasing pressure from ever expanding urbanisation and ever present threat of biological invasion. Self-fertilisation is a reproduction strategy that is readily employed by this species, and one which is beneficial when individuals may be scattered and less able to reach a mate in novel environments such as man-made structures. The increase in abundance surveyed from this study compared to the survey carried out a decade ago (Schiel & Hickford 2001) may suggest that this species is thriving and not showing any adverse signs from urbanisation or invasion. This is not to say that urbanisation and invasion pressure is non-existent. Urbanisation is increasing around the Banks Peninsula and the threat of invasion is ever present from the high volume of shipping traffic in the area. However, currently the populations of *Pyura*

pachydermatina seem capable of resisting the changes and disturbances from urbanisation and invasion.

This study provides a basis for additional investigations relating to the resilience of native species. I have shown that the reproductive strategy of *P. pachydermatina* affords it some resilience to invasive threats due to a constant presence of multiple cohorts. However, it is also clear that predation from invasive amphipods is a very real and current threat to this species, for which additional studies are required to assess the impacts on the populations of this precious Taonga species and other native species within the coastal community.

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Appendix

Table A1. Analysis of deviance table generated from R for GLM on abundance data with site, season, and site+season as the factors.

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			406	2011.9		
site	2	817.13	404	1194.7	134.264	<2e-16 *
season	3	1.49	401	1193.2	0.163	0.9212
site:season	6	9.75	395	1183.5	0.534	0.7825

Table A2. Model simplification tested for any loss of explanatory power after taking out non-significant factors by using 'anova' function in R.

Factor	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)
site:season	395	1183.5				
	401	1193.2	-6	-9.7491	0.534	0.7825
season	395	1183.5				
	404	1194.7	-9	-11.237	0.4103	0.9296

Table A3. Analysis of deviance table generated from R for GLM on size data with site, season, and site+season as the factors.

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			3449	22127		
Site	2	2112.46	3447	20015	211.5295	< 2.2e-16*
Season	3	1114.31	3444	18900	74.3870	< 2.2e-16*
Site:Season	6	151.65	3438	18749	5.0616	3.517e-05*

Table A4. Multiple pairwise *t* test *post-hoc* analysis of mean lengths in all sites over four seasons showing significance or non-significance in mean size differences. (CB=Camp Bay, PB=Pigeon Bay, Wa=Wainui, Su=Summer, F=Fall, W=Winter, Sp=Spring, SIG= significant ($p<0.05$), and NON= not significant ($p>0.05$))

	CB.Su	CB.F	CB.W	CB.Sp	PB.Su	PB.F	PB.W	PB.Sp	Wa.Su	Wa.F	Wa.W
CB.F	NON	-	-	-	-	-	-	-	-	-	-
CB.W	SIG	SIG	-	-	-	-	-	-	-	-	-
CB.Sp	SIG	SIG	NON	-	-	-	-	-	-	-	-
PB.Su	SIG	SIG	NON	NON	-	-	-	-	-	-	-
PB.F	SIG	SIG	NON	NON	NON	-	-	-	-	-	-
PB.W	SIG	SIG	SIG	SIG	SIG	SIG	-	-	-	-	-
PB.Sp	SIG	SIG	SIG	SIG	SIG	SIG	NON	-	-	-	-
Wa.Su	SIG	SIG	NON	NON	NON	NON	NON	SIG	-	-	-
Wa.F	SIG	SIG	SIG	SIG	SIG	SIG	NON	NON	NON	-	-
Wa.W	SIG	SIG	SIG	SIG	SIG	SIG	SIG	SIG	SIG	SIG	-
Wa.Sp	SIG	SIG	SIG	SIG	SIG	SIG	SIG	SIG	SIG	SIG	SIG

Table A5. Classification and description used to determine *Pyura pachydermatina* gonad developmental stages.

Stage		Ovaries	Testes
1	Inactive	Ovaries small and compact, consists of inactive germ cells. Central lumina absent.	Testis lobes small. Germinal epithilia consist only of germ cells.
2	Early development	Ovaries contain some ova in addition to various egg maturational stages. Central lumina are lined by germinal epithelium.	Testis lobes convoluted, possess thick germinal epithilia composed of spermatogonia and spermamtocytes. Some spermatozoa aggregates found in central lumina.
3	Late development	Ovaries contain mainly ova and oocytes in the final maturational stages. Central lumina are lined by discontinuous layer of germinal epithilia.	Large central lumina due to accumulation of spermatozoa aggregate. Germinal epithilia have a patchy appearance.
4	Morphologically ripe/ mature	Ovaries swollen laterally and longitudinally with ova. Germinal epithilia no longer active, being reduced to the occasional germ cells lining the central lumina.	Germinal epithilia reduced to a few-cell thickness. Testis lobes appear swollen, full of spermatozoa aggregates, and appear dense.
5	Spawned/ redeveloping	Ovaries large and slack, containing few ova. Central lumina active and line with layers of thick germinal epithilia.	Large spaces are visible in the central lumina with very few spermatozoa.

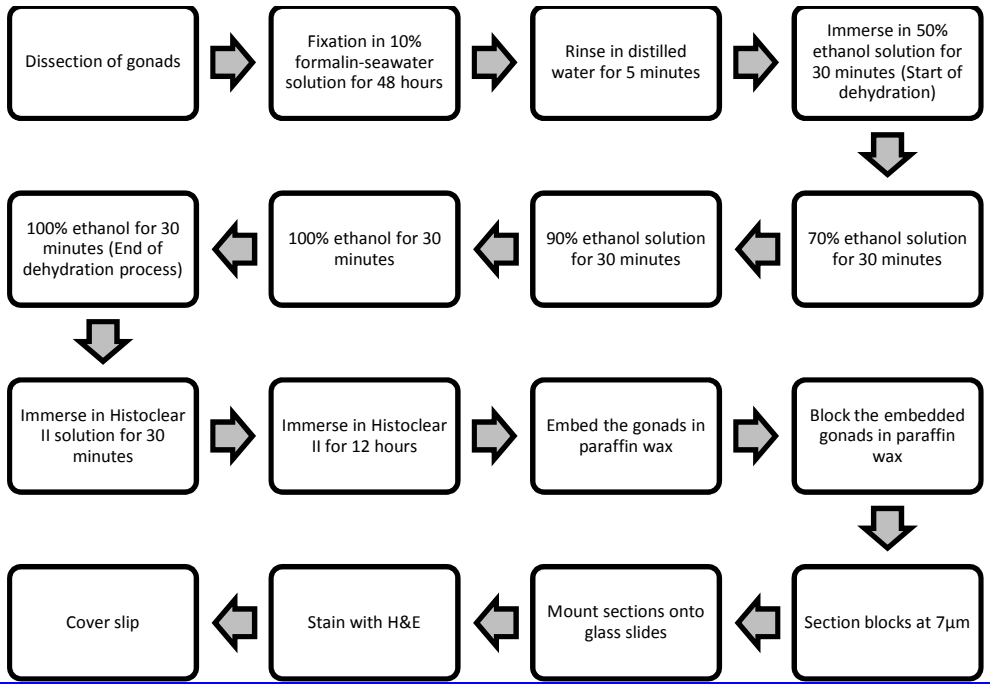

Figure A1. Histology processing workflow of *Pyura pachydermatina* gonads.